STUDIES ON DISEASE COMPLEX OF SOYBEAN *(Glycine max)* INVOLVING ROOT-KNOT NEMATODE AND FUNGI.

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Dedicated

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Certificate

This is to certify that the dissertation entitled "**Studies on Disease Complex of Soybean *(Glycine max)* involving Root-knot Nematode and Fungi**" submitted to the department of Botany, Aligarh Muslim University, Aligarh in partial fulfilment for the degree of ***Master of Philosophy in Botany (Plant Pathology)*** is a faithful record of the bonafide research work carried out by ***Miss Priyanka Singh*** under my supervision.

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Introduction

INTRODUCTION

Soybean is called as miracle bean in India. Now it is not only a leading crop but also one of the most important and rapidly increasing oilseed crop in the world.

Soybean belongs to family Leguminosae, sub-family Papilionoidae (Fabaceae) and genus *Glycine*. It is mainly sub-tropical plant but cultivation extends, however, to tropical temperate regions upto 52°N latitude.

Soybean contains 40% protein and 20% oil. It is a rich source of Calcium and vitamin 'A'. In calories it is similar to other legumes. Soybean oil contributes about 30% of the total world production of edible vegetable oils. Soybean is also, the best and cheapest source for high quality vegetable protein that is equivalent to meat, milk products and eggs in quality.

Meloidogyne (Kofoid and White, 1928) is one of the most important disease causing phytonematode in tropical and subtropical regions of the world. It causes root-knot disease by attacking the under-ground parts of the plants, where they induce the development of abnormal growth of the roots. Sometimes large galls are developed at the base of the stem. The economic damages caused by a single species *M. incognita* range from less

than 1% to total loss of the crop. More than twenty crops of cereals, pulses, fruits and vegetables are destroyed by various species of *Meloidogyne,* every year.

Meloidogyne incognita is a sedentary root endoparasite. It completes its life cycle within 30 days. The root-knot nematode infection causes disruption of xylem and phloem tissues resulting in hindrance in the transportation of water, mineral nutrients and translocation of food materials in the host plants. The formation of abnormal xylem has been reported on different plants infected with root-knot nematodes (Littrell, 1966; Siddiqui and Taylor, 1970; Siddiqui et al., 1974; Byrne et al., 1977; Mean et al., 1978; Jones, 1981; Pasha et al., 1987).

On soybean roots *M. incognita* infection caused hypertrophy, hyperplasia and giant cell formation in the tissues surrounding the head that consequently lead to gall formation (Ibrahim and Massoud, 1974). According to Siddiqui and Taylor (1970) gall formation is attributable to hypertrophy of the cortical cells, xylem parenchyma, formation of giant cells, nematode development and egg mass production.

Among the fungal diseases, *Fusarium* wilt is one of the most serious disease worldwide. *Fusarium* spp. occur frequently

in both temperate and tropical regions on a wide variety of substrates. Wilting is incited by specific formae specialis of *Fusarium oxysporum* that infect many cultivars and species of plants. *Fusarium* spp. are facultative parasites and primarily survives in the soil but when suitable host plants are sown in such a soil, they attack them and start living as parasites. Formae specialis of *Fusarium oxysporum* are vascular parasites and are thus often referred to as vascular fusaria. Often the first indication of wilting is that the lower leaf petioles bend downwards so that the angle between them and the main stem becomes obtuse (epinasty). Sometimes slight vein clearing occurs followed by the yellowing of the lower leaves. Vascular browning and the development of tyloses and gums has been observed. Ultimately the plant wilts, shrivels and dies.

The fungus present in the soil invades the root cortex usually without damaging it to any great extent, and then becomes established in the vessels. Later when the host plant is dying it grows out into the cortical tissues and then it may spread to adjacent healthy plants through root contact.

The wilting may be associated with increased levels of indolacetic acid, production of ethylene and enzyme activity.

However, plugging of xylem vessels that interferes with minerals and water uptake of plant and production of toxins by the fungus are the two principle causes of wilting.

Another important fungal disease is root rot disease, caused by members of the genus *Pythium*, present in agricultural and forest land all over the world. In fact, species of *Pythium* constitute one of the most destructive groups of plant pathogens. Many of them are soilborne pathogens, capable of causing serious economic losses on a wide range of hosts. Disease symptoms appear on roots in the form of localized necrotic lesions that spread over the root system which decays. The parasitic species kill the host and continue to live as saprophytes on the remains of the host. Plant infection by *Pythium* spp. may lead to yield reduction or death of plants.

Microorganisms often develop symbiotic, synergistic or antagonistic relationship amongst themselves which could primarily be because of nutritional or spatial competition. All living organisms either cooperative or compete with the other especially when they have similar and overlapping food resources. The plants in one or the other form, are the direct or

indirect source of food for consumers of all tropic levels, including plant pathogenic organisms.

It is well known that plants are exposed to various pathogens, instead of a single pathogen. Fawcett (1931) recognized that nature does not work with pure cultures and that many plant diseases are influenced by associated micro-organisms whose action is often combined to induce damage. Mostly pathogens such as fungi, nematodes, bacteria and viruses are quite capable of causing serious diseases without being influenced by other biotic agents, but the economic damage often becomes more destructive and high when they interact with each other (Powell and Nusbaum, 1960; Powell, 1968; Johnson and Powell, 1969; Husain et al., 1985; Weischer, 1983; Evans and Haydock, 1993; Sitarmaih and Pathak, 1993; Francl and Wheeler, 1993; Shahzad et al., 1995; Chahal, 1998 and Patel et al., 2000).

Besides the direct damage caused to the plant, the root-knot nematodes are notorious for the disease complexes involving fungi, bacteria, viruses, mycoplasma, insects and other nematodes. The most important of these are the complexes formed by the root-knot nematodes and the root invading fungi causing wilt and root-rot diseases as have been reviewed by

Powell (1971), Taylor (1979), and recently by Khan (1984) and Webster (1985).

Of all the interactions of pathogens with nematodes, none are as damaging to crops world wide as the combined effects of wilt inducing fungi and plant parasitic nematodes. The combination of nematodes and fungus often results in a synergistic interaction whereas the crop loss is greater than expected from either pathogen alone or an additive effect of the two together. Keeping in view the role played by the parasitic nematodes in disease complexes the following experiments were conducted and the results of which embodies in the present dissertation:

1. Effect of different concentrations of culture filtrates of *Fusarium oxysporum* and *Pythium aphanidermatum* on the hatching of IInd stage larvae of *Meloidogyne incognita*.
2. Effect of different concentrations of culture filtrates of *Fusarium oxysporum and Pythium aphanidermatum* on the mortality of IInd stage larvae of *Meloidogyne incognita*.
3. Effect of different inoculum levels of *Meloidogyne incognita* on plant growth, number of pods, chlorophyll

contents, nematode development, nodulation, and gall formation on *Glycine max* var. L.

4. Effect of different inoculum levels of *Pythium aphanidermatum* on plant growth and number of pods, chlorophyll contents, nodulation and root-rot development on *Glycine max*. var L.

5. Effect of different inoculum levels of *Fusarium oxysporum* on plant growth and number of pods chlorophyll contents, nodulation and wilt index on *Glycine max*. var. L.

6. Effect of individual, sequential and simultaneous inoculations of *M. incognita*, *Pythium aphanidermatum and Fusarium oxysporum* on plant growth, number of pods, chlorophyll contents, nematode development, gall formation, nodulation, root-rot and wilt-index on *Glycine max*. var. L.

Review of Literature

REVIEW OF LITERATURE

Disease complexes involving nematodes and other micro-organisms are common in nature as plants are always exposed to large number of pathogenic microorganisms both in aerial and soil environment. Thus plants, their pathogenic organisms and the surrounding environment form a triangle which naturally can involve various types of associations or interactions. Soil is a complex ecosystem, inhabiting a wide variety of life forms, pathogenic as well as saprophytic, which often show synergistic, symbiotic and antagonistic relationship between themselves. Such associations may be beneficial or deleterious to plants. Disease syndrome resulting from root diseases are often caused by microbial interactions. Plant parasitic nematodes have principal role in many interactions, usually making plant roots more susceptible to invasion and parasitism by other soil inhibiting microorganisms (Hussey and McGuire, 1987). Several possibilities have been suggested by different workers by which plant parasitic nematodes interact with other microorganisms. Root-knot nematode causes greater damage in natural soil than in autoclaved soil (Mayol and Bergeson 1970; Starr and Mai, 1976). Many species of plant-parasitic nematodes predispose the plants to

fungal and bacterial infections and thus the plants may suffer greater damage from concomitant infection. According to Pitcher (1965), nematodes may act as (i) vectors of pathogens capable of self establishment once in contact with the host (ii) vectors of pathogens incapable of self-establishment unless introduced below the host epidermis (iii) mechanical wound agents, (iv) providers of necrotic infection – courts, (v) modifiers of the substrates, (vi) breakers of disease resistance and (vii) deterrents of plant diseases. The literature on this subject has been extensively reviewed by several authors (Taylor, 1990; Khan, 1993; Sobita, 1995; Hillocks et al., 1996; Khan, 1993; Sobita, 1995; Hilloctis et al., 1996; Fazal et al., 1998; Mahapartra et al., 1999; Patel et al., 2000) which firmly establish the involvement and role of phytoparasitic nematodes in interactions with other microorganism.

Nametode – Fungus Interactions:

Interaction between nematodes and fungi have been recognized since 1892. Atkinson reported that Fusarium wilt (*Fusarium oxysporum f. sp. vasinfectium*) (Atk.) Synder and Hansen) was more severe in presence of root-knot nematodes (*Meloidogyne* spp.) than in its absence. Recently several workers

have reviewed the work on interactioin of phytoparasitic nematodes with fungi an various crops (Pitcher, 1978; Powell, 1971 a, 1979; Bergeson, 1972; Riedal, 1988; Hasan, 1993; Franchl and Wheeler, 1993; Evans and Haydock, 1993; Swain and Kar, 1994; Krishna and Krishnappa, 1996; Chandal et al., 1998; Erma and Shahzad, 1998). Nematode-fungus interactions have been classified in number of ways and the roles played by nematodes in such interactions have been examined thoroughly. Powell (1971a) categorised the nematode-fungus interactions on the basis of symptomotology of disease caused by fungi into following three types:

1. Nematode – fungus wilt disease interactions.
2. Nematode – fungus root-rot disease interactions.
3. Nematode – fungus seedling disease interactions.

Although the involvement of nematode-fungus disease complex situation is widespread and the literature is quite extensive, but in the present study the review of literature is confined to the interactions involving nematodes and wilt fungi and nematode and root-rot fungi.

1) Interaction with wilt causing Fungi:

The interaction between *Meloidogyne* spp. and wilt causing fusaria have been studied more than any other nematode fungus combinations (Powell, 1963, 1971; Mai and Abawi, 1987; Francl and Wheeler 1993). Interaction between *Meloidogyne* spp. and *Fusarium* spp. causing wilt has been studied by different investigators on cowpea (Thomson et al., 1959) on chickpea (Mani and Sethi, 1987) on black gram (Swain and Kar, 1994, Sankaranarayanan and Sunerbabu, 1998) on soybean (Goswami and Agarwal, 1979).

Much work has been done on the interaction of wilt causing fungi with endoparasitic nematodes particularly *Meloidogyne* spp. as compared to semi-endoparasitic or ecto-parasitic nematodes (khan, 1984; Francl and Wheeler, 1993). The interaction of nematodes with wilt causing fungi especially *Fusarium* spp. has drawn much attention (Powell et al., 1971; Franchl and Wheeler, 1993).

Root-knot nematode (*Meloidogyne* sp.) forms an important relationship with *Fusarium* wilt Root-knot nematode infection predisposed tomato plant to *F. oxysporum* f.sp. *lycopersici* that was normally virulent in green house tests (Goodie and McGuere,

1967). Noguera (1983) also suggested that *M. incognita* infested tomato roots became generally susceptible to invasion by *F. oxysporum*. A number of studies on interaction of root-knot nematodes and wilt causing fungi have been conducted on leguminous plants. In a study McGuire et al., (1958) observed highest wilting percentage of alfalfa variety Buffalo plants when inoculated with root-knot nematode, *M. hapla* and *Fusarium oxysporum* f. sp. *vasinfectum*. Thomson (1958) found that wilt of *Vigna sinensis* was more severe in plants grown in soil infested with both *F.oxysporum* and *M. javanica* than in soil infested with fungus alone. Mousa (1997) observed that the interactions of the fungi and the root-knot nematodes resulted in a great deal of damage to soybean plants. Wilt of soybean developed dramatically when the *M. javanica* were introduced to the soil as compared to the treatment of the fungi *Fusarium oxysporum* f.sp. *glycines* alone. Ross (1965) observed that certain varieties of soybean, inoculated with *Fusarium* fungus and *Heterodera glycines*, wilt to a greater extent than those predisposed by *M. incognita*. McGuire et al., (1958) observed that only 10% plants showed the wilt symptoms when infected with *M.incognita acrita* and 15% with *F. oxysporum* f. sp. *vasinfectum* alone, where as 95%, 60% and 50% plants became wilted when the fungus was present in

combination with *M. hapla*, *M. javanica* and *M. incognita* respectively.

Presence of the nematode enhanced the development and severity of wilt. Padila et al., (1980) observed that *Meloidogyne* spp. alone caused severe stunting, *F. oxysporum*. alone caused no detrimental effect, where as concomitant inoculation of the nematode and fungus at planting caused the death of the plants after 45 days. Khan and Salam (1990) observed synergistic interaction between *M. javanica* and *F. udum* on pigeonpea. Wilt symptoms appeared earlier and more severe than in plants inoculated with the fungus alone. Interaction of root-knot nematodes and wilt causing fungi on chickpea have also been studied by some workers. Inoculation of chickpea either with *M. incognita* or F. *oxysporum* f.sp. *ciceri* reduced growth of chickpea and highest reduction was obtained with simultaneous inoculation of both the pathogens followed by the treatment in which nematode inoculation preceded the fungus by ten days (Kumar et al., 1988).

Mani and Sethi (1987) studied the effect of combined inocula of *M. incognita* and *F. oxysporum* or F. *solani* on the growth of chickpea which was found to be additive in nature.

However, when nematode was established earlier than the two fungi, the resultant effect was more than additive. Occurrence of *M. incognita* in combination with F. *oxysporum* f. sp. ciceri and *F. solani*, not only increased the severity of disease but also shortened the incubation period for disease expression. Patel et al., (1987) also observed increase in the incidence of chickpea wilt due to *F. oxysporum* f. sp. *ciceri* in presence of *M. incognita*. In pot experiments Upadhyay and Dwivedi (1987) found highest wilt symptoms in chickpea plants inoculated simultaneously with both M. *javanica and F. oxysporum f.sp. ciceri,* followed by severity by inoculations of the nematode preceding the fungus and fungus preceding the nematode. The maximum number of root -galls were recorded on roots inoculated with nematode alone and the minimum number on roots where inoculation of the fungus preceded the nematode. The maximum reduction in shoot weight occurred where inoculation of nematode preceded that of the fungus. Khan and Hosseini Nejad (1991) observed synergistic interaction between *M. javanica* and *F. oxysporum* f. sp. *ciceri* on chickpea cultivers both in concomitant and sequential inoculations. Wilt symptoms were most prominent in the presence of *M. javanica* in concomitant inoculation than in sequential ones.

Resistance of Pusa-212 recorded with the inoculation of fungus alone, was broken in the presence of the nematode.

Goswami and Agrawal (1979) reported the interaction between species of *Fusarium* and root-knot nematode *M. incognita* in soybean. Pot experiments were conducted to determine the relationship between *M. incognita* and *Fusarium oxysporum, F. solani, F. graminearum* and *F. equiseti* on soybean. Seedlings were inoculated with nematode and fungus alone, simultaneously or with one organism preceding the other by 3 weeks. About 45 days after inoculations, plant roots and shoots were measured and weighted and nematode galls counted. The results showed an antagonistic interaction of *F. oxysporum, F. solani with M. incognita.* The fungus when established first suppressed nematode multiplication, while the nematodes when established first reduce symptoms due to the fungus. The interaction of *F. graminearum* and *F. equiseti* with *M. incognita* was synergistic, nematode – infested plants being more severely damaged by the fungi than the non-infected plants.

Root-knot nematodes break the resistance of crop plants against soil borne pathogens. This aspect has been reviewed recently by Mai and Abawi (1987), Hasan (1993) and Prot (1993).

Harrison and Young (1940) observed that root-knot nematode reduced the wilt resistance of several varieties of tomato in glasshouse experiments. Twenty tomato lines and varieties inoculated with wilt fungus and root-knot nematode, more found to be susceptible including those resistant to *Fusarium oxysporum. f.sp. lycopersici.*

Mouso and Hague (1988) studied the influence of the root-knot nematode, *Meloidogyne incognita*, on the development of *Fusarium oxysporum* f.sp. *glycines* in wilt resistant and wilt susceptible soybean cultivars. Exposure of Amsay (wilt susceptible) and Coll (wilt resistant) soybean to *Fusarium oxysporum* f.sp. *glycines* in the presence of the nematode resulted in a large increase in the amount of the fungus found in the vascular tissues of the roots and stems as compared to the amount found in plants exposed to the fungus alone. The fungus was isolated from leaves and seeds of the wilt resistant cultivar only when the nematode was present. Mouso and Hague (1989) also observed that fungus *Fusarium oxysproum* f.sp. *glycines* play an important role on the invasion and development of *Meloidogyune incognita* on soybean. When *M. incognita* and wilt fungus *F. oxysporum*. f.sp. *glycines* were inoculated simultaneously onto growing seedlings of the soybean cultivars Ware and Coll, nematode invasion of the

root was not affected but giant cells were invaded by the fungus and destroyed. The over all effect was to reduce the number of females of *M. incognita* and increase the proportion of males.

Dwivedi et al., (1992) studied the interaction between the root - knot nematode, *Meloidogync incognita* and the fungus *Fusarium oxysporum* f.sp. *udum* on pigeonpea var. T-21 in pot condition. The results indicated that the effect of the nematode in combination with the fungus enhanced the suppression of growth of plants including bacterial nodules. Of the two organisms *M. incognita* affected the plant growth characters to a greater extent in comparison to fungus, however, maximum growth reduction was observed when, both the organisms were present at higher level. The bacterial nodulation was adversely affected in the presence of both the organisms. The nematode development and multiplication was also affected by the presence of fungus and maximum gall index was found at higher inoculum level.

Fazal et al., (1994) reported the interaction between *M. incognita* and *Fusarium oxysporum* f.sp. *lentis* on Lentil using various combinations of each pathogen. Individually, F. *oxysporum* f.sp. *lentis* was the most aggressive pathogen. At all combinations, reduction in growth parameters in concomitant

inoculation was greater than the additive of the pathogens acting independently, thus showing a synergistic relationship. Nematode multiplication and galling in the presence of fungus were significantly reduced.

Fazal et al., (1994) studied the effect of individual and combined inoculations of *Meloidogyne javanica, Rhizoctonia solani* and *Fusarium oxysporum f.sp. ciceri* on chickpea in various combinations. Singly *F. oxysporum f. sp. ciceri* was the most aggressive pathogen. In concomitant inoculations, reduction in growth parameters was greater than the sum total of reductions caused by the pathogens alone. All the three pathogens adversely affected nodulation singly as well as in concomitant inoculations, and reduction in nodulation followed a similar trend in plant growth. Rate of nematode multiplication and galling was significantly reduced in the presence of both the fungi.

Swain and Kar (1994) reported the disease complex involving *M. incognita* and *F. pallidoroseum* on black gram. Wlting was maximum in treatments receiving *M. incognita* 7 days before *F. pallidoroseum* followed by simultaneous inoculation of both the pathogens. Multiplication of *M. incognita* was adversely affected by *F. pallidoroseum*. Devi Sobita (1995) studied the

pathogenicity of *Rotylenchulus reniformis, Meloidogyne incognita and Pratylenchus thornei and their interactive effect with Fusarium oxysporum f.sp. ciceri* on chikpea.

Sheela (1990) observed that the inoculation with *M. incognita* and *F. oxysporum* on black pepper had a synergistic effect on growth retardation. She observed that the fungal infection in stem portion was more severe in plants with prior nematode inoculation than that in simultaneous inoculation, or fungus prior followed by nematode respectively.

France and Abawi (1995) studied the interaction between *Meloidogyne incognita* and *Fusarium oxysporum* f.sp. *phaseoli* on selected bean genotypes. Four bean genotypes (IPA-1, A-221, A-107 and Calima), representing all possible combinations of resistance and susceptibility to *F. oxysporum* f.sp. *phaseoli* (Fop) and *M. incognita,* were each inoculated with three population densities of these pathogens. In Fop - susceptible lines (IPA-1 and A-211), the presence of *M. incognita* contributed to an earlier onset and increased severity of *Fusarium* wilt symptoms and plant stunting. However, the Fop resistant Calima developed symptoms of Genotype A-107 resistant to both (M. incognita and Fop) and exhibited Fusarium wilt symptoms and a moderately susceptible

reaction to Fop only after the break down of its *M. incognita* resistance by elevated incubation temperature (27^{0}C). Root galling and reproduction of *M. incognita* were increased on the *M. incognita* susceptible cultivars. However, these factors were decreased as the inoculum density of Fop was increased. It was concluded that severe infection of bean roots by *M. incognita* increased the severity of *Fusarium* wilt on Fop-susceptible genotype and may modify the resistant reaction to Fop.

Hillocks and Marley (1995) observed the systemic effect of root-knot nematodes on mechanism of resistance to *Fusarium* wilt disease. Root-knot nematode *(Meloidogyne spp.)* induced profound changes in the structure and function of the xylem tissues in which they feed. These changes are associated with increased incidence of *Fusarium* wilt in number of crops. One or more mechanism may operate to increase the plants susceptibility to infection, depending on the host and nematode species involved. Systemic effects on host susceptibility to *Fusarium* wilt diseases have been indirectly demonstrated for some crops, although in most cases the mechanism of the effect is unknown. For cotton and pigeonpea, a partial breakdown in resistance occurs in the presence of root-knot nematode and this is attributed to nematode induced decreases in the effectiveness of vascular

occlusion in cotton and to a retarded phytoalexin response in pigeon-pea.

Rao and Krishnappa (1996) discussed the wilt disease complex of chickpea caused by *Meloidogyne* spp. and *Fusarium* spp. in different agroclimatic zones of Karnataka. Rao and Krishnappa (1996) again reported the interactioin of *F. oxysporum* f.sp *ciceri* with *M. incognita* on chickpea cultivar Annagui in two soil types. Inoculation of chickpea with fungus, either with nematodes or seven days before or after nematode inoculation resulted in significant reduction in fresh weight and dry weight of shoot and fresh weight of roots as well as an increase in wilt incidence as compared to the plants inoculated with the fungus alone. Nematode multiplication and root-knot incidence were higher in the alfisol and fungal growth and wilt incidence were higher in the vertisol. Krishna and Krishnappa (1996) also studied the wilt disease in chickpea cultivars caused by *M. incognita and Fusarium oxysporum* f.sp. *ciceri.*

Rao and Krishnappa (1997) reported a preliminary survey of chickpea crop. They studied the occurrence of the *Meloidogyne-Fusarium* pathogen complex associated with chickpea, during Nov, Dec, 1991 in 15 districts in Karnataka, India. Data for each

location on total wilted plants and population densities of *Meloidogyne* and *Fusarium* in root and soil samples indicated co-infection of *Meloidogyne* spp. and *Fusarium* spp. in 13 districts. In addition to *Meloidogyne* spp. other plant parasitic nematodes *(Heterodera, Helicotylenchus, Hoplolaimus, Pratylenchus, Rotylenchulus, Tylenchorhynchus* and *Aphelenchoides)* were found associated with wilted chickpea. *F. oxysporum* f.sp. *ciceri* and *F. solani* were the predominant fungi found associated with wilted plants but *R. solani, R. bataticola (Macrophomina phaseolina), Verticillium* spp. and *Sclerotium rolfsii (Corticum rolfsii)* were also detected.

Rao and Krishnappa (1997) conducted a survey on prevalence of *Meloidogyne-Fusarium* wilt disease complex of chickpea in Karnataka. They also observed in 1997 effects of the interaction of *Meloidogyne incognita* and *Fusarium oxysporum* f.sp. *ciceri* on root-knot disease and wilt incidence in chickpea cultivars susceptible to *M. incognita*. Root-knot index was lowers in the presence of *F. oxysporum* f. sp. *ciceri*. There was an increase in wilt incidence in the presence of nematode and 2 wilt resistant cultivars B Dal-9-3 and Aurodhi lost their resistance in the presence of *M. incognita*.

Effect of rhizosphere fungi and root-knot nematode on mungbean *(Vigna radiata)* variety ML-131 under the pot culture conditions was reported by Chahal et al, (1997). Simultaneous infection of *M. incognita* and pathogenic fungi *(Fusarium oxysporum* and *Macrophomina phaseolina)* caused more damage to the plants than individually.

Variability among *F. oxysporum* f.sp *lycopersici* isolates in their ability to interact with *Meloidogyne incognita* race-1 was observed by Suleman et al., (1997). Isolates of *F. oxysporum* f. sp. *lycopersici* obtained from different locations, were studied to determine that different populations of the fungus varied in their ability to form a disease complex with the root-knot nematode. All race-1 isolates of *Fusarium oxysporum* f.sp. *lycopersici* were similar in being unable to overcome monogenic resistance in tomato during co-infection with nematodes. Isolates were also similar in causing a greater severity of wilt and vascular discolouration during co-infection of tomato with nematodes. Individual isolates differed with regard to the increase in wilt symptoms severity and extent of vascular discolouration induced during co-infection, suggesting possible heterogeneity among *F. oxysporum* populations in the degree of interaction with *M. incognita*.

Charu and Trivedi (1988) reported the effect of interaction between *Meloidogyne incognita, Fusarium oxysporum* and *Rhizoctonia bataticola* on chickpea. A combination of root-knot nematode, *Meloidogyne incognita* with *F. oxysporum* and *R. bataticola* (*Macrophomina phaseolina*) caused more severe disease and yield losses in chickpeas. All the three pathogens were inoculated in different combinations to assess their role in disease severity. Reduction in plant growth, severity of root-knot and wilt incidence were greater in the combined treatments as compared to the pathogens alone. The reduction in shoot, root, length and weight was more pronounced when *F. oxysporum, Macrophomina phaseolina, R. bataticola* and *Meloidogyne incognita* were combined together followed by each fungus with nematode as compared to control and the each fungus alone.

Satyendra and Goswami (1999) observed penetration and development of root-knot nematode, *Meloidogyne incognita* alone and in presence of wilt fungus, *Fusarium oxysporum* in susceptible and resistant cultivars of cowpea.

Patel et al., (2000) observed interaction between *Meloidogyne incognita* and wilt inducing fungus, *Fusarium oxysporum* f.sp. *ciceri* on chickpea cv. Dahod Yellow and

revealed that the organisms either individually or in combinations reduced plant height and fresh root and shoot weight significantly but the reduction was more by *Meloidogyne incognita* as compared to *Fusarium oxysporum* f.sp. *ciceri*. Among combined inoculations, simultaneous inoculation of both the pathogens had maximum suppressive effect on growth of chickpea plants as compared to preceding or succeeding inoculation of fungus and nematodes. Root galling and nematode multiplication on chickpea were maximum when nematodes were inoculated alone but it was reduced in the presence of fungus. The fungus alone was able to produce wilt disease but the incubation period for disease development was reduced and severity of the disease increased when root-knot nematode was present with fungus. Maximum wilting of plant was observed when the fungus and nematodes were inoculated simultaneously.

Similarly, in tomato, interaction of *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *lycopersici* was reported by Bhagwati and Goswami (2000). Starr (1991) evaluated twenty one cultivars of cotton for their response to *F. oxysporum f. sp vasinfectum* and *M. incognita* in field microplots. He too noticed that mortality and disease incidence was greater in the presence of the nematode then when the nematode was absent. Kafagi et al.

(1992) inoculated nine strawberry cultivars with *M. incognita* or *M. javanica* alone or in combination with *F. oxysporum f. sp. fragariae*. Highest reduction in plant dry weight was observed in plants inoculated with *M. javanica* and *F.* oxysporum simultaneously. The nematode enhanced the development and severity of wilt disease. The severity of *Fusurium* wilt in chrysanthemum vr. Iceberg. Was enhanced in the presence of *M. javanica/M. incognita/ M. hapla* (Johnson and Littrell, 1969).

Singh and Goswami (2001) reported the interrelationship between *Meloidogyne incognita* and *Fusarium oxysporum* on susceptible and resistant cultivars of cowpea. *Meloidogyne incognita* enhanced wilting of cowpea Pusa Komal when inoculated in combination with *Fusarium oxysporum*. Nematode inoculation preceded by fungal inoculation showed maximum effect (synergistic), followed by the treatment when both the pathogens were inoculated simultaneously. Presence of nematodes not only predisposed the host but also shortened the incubation period for disease expression. Both the pathogens interacting simultaneously or nematode infecting prior to fungus, affected nodulation.

2. Interaction of root-knot nematode and root-rot fungus:

The frequency of involvement of nematodes and fungi in diseases complexes is reflected in number of crops on which such complexes are recorded and amongst the different plant parasitic nematodes of economic importance the root-knot nematodes (*Meloidogyne* spp.) have been thoroughly studied and commonly found involved in synergistic interactions with root-rot fungi. The root-rot fungi constitute a category of pathogens where considerable work has been carried out with respect to their interactions with nematodes. Prominent amongst the root-rot fungi are species of *Rhizoctonia (R. solani, R. bataticola* (Taub.) Butler), *Pythium* (*P. aphanidermatum* (Eds). Fitz., *P. ultimum* Throw.), *Fusarium* (*F. solani* (Mart.) App. Wollenw), *Phytopthora parasitica* Dastur, *Sclerotium rolfsii* Sacc., and Colletotrichum coccades. (Wallr) Hughes that are known to interact with different plant parasitic nematodes. The role of nematodes in root-rot diseases, in general, is related to assisting the fungal pathogen is its pathogenesis and increasing host susceptibility.

A number of studies underline the significance of nematode infected tissues from which the fungal pathogen derives

aggressiveness and became pronounced. The root-knot nematode, *M. incognita* have been found to predispose the plant roots for secondary infection (fungal attack) resulting in the greater damage of plants by way of root decay. The disease caused by fungal pathogens become more pronounced and may appear earlier when plants are infected with nematodes.

Pythium is one of the fungus which causes seed rot, damping-off of seedlings and root-rot of all types of plants. It also causes soft rots of fleshy fruits. It is likely that the interactive effects of *P. aphanidermatum* and *M. incognita* may be different to lesser or greater extent than their individual effects, on growth and productivity.

Steiner (1942) for the first time realized and discussed the importance of root-knot nematode with respect to plant and root-rot fungi. Since then large number of workers have studied the association between root-knot nematodes and root-rot fungi on different crops.

Powell et al., (1971) showed that when tobacco roots had been previously infected by *M. incognita*, non-pathogenic soil inhibiting fungi became pathogenic and galled roots were extensively decayed following invasion by these fungi. Powell and

Batten (1967) and Melendez and Powell (1969, 1970), reported that nematodes may predispose plants to many fungal disease. If plants were previously invaded (three or four weeks) by *M. incognita* then both *Pythium ultimum* and *Rhizoctonia solani* were found to be capable of invading the roots and causing rapid necrosis. The necrosis failed to develop in varieties resistant to root-knot nematodes. Nava (1970), reported that tomatoes respond very similarly to tobacco. He found decay in tomato roots only when *M. incognita* was added several weeks ahead of either of fungus *Rhizoctonia solani* or *P. ultimum*. In the roots, where both *R. solani* and *P. ultimum* were present along with *M. incognita*, the *R. solani* appeared to be more aggressive than *P. ultimum* and gradually become dominant in the galled tissue.

Littrell and Johnson (1969) and Johnson and Littrell (1970) observed that root-knot nematodes are important in root decay complexes. *Meloidogyne incognita* interacts with *P. aphanidermatum* and promotes root destructions of chrysanthemum plants as chrysanthemum roots inoculated with *M. incognita* and *P. aphanidermatum* developed symptoms of *Pythium* root-rot earlier and more extensively than those of inoculated with fungus alone.

Decay does not develop significantly when plants are inoculated with the nematode or either the fungus separately. Melendez and Powell (1970) carried out histopathological studies with the root-knot and *Pythium* complex in tobacco. Fungal penetration of galled and non-galled areas of nematode infected roots, as well as nematode free roots, have been studied from initial penetration through colonization. There is no appreciable invasion of roots free from nematodes. However, both galled and non-galled regions of roots infected by *M. incognita* are readily invaded by *P. ultimum* and colonization is virtually complete only after six days.

James and James (1977) reported early development of *P. plymorphon* on celery roots infected by *M. hapla*. Root galls were colonized by the fungus 24h after inoculation, but invasion of *M. hapla* free roots was not noticed until 48h. A greater percentage of galled than of non-galled root segments was colonized at 72 h. After ingress, *P. polymorphon* colonized both galled and non-galled root segments at apparently similar rates. The fungus seemed to invaded non-galled portions of galled roots from infected galls. Lanjewer and Shukla (1985) reported that rotting of ginger roots by *P. myriotylum* was equally severe in the presence or absence of *M. incognita* but the presence of fungus decreased

nematode reproduction. Hasan (1985), reported *Pythium aphanidermatum* and *Rhizoctonia solani* were both found to interact with *M. incognita* on chilli, causing extensive damage to them.

Besides root-knot nematodes, species of *Phythium* interact with other nematodes. Members of the genus *Pratylenchus*, are involved in certain root rotting complexes. A condition commonly known as "peach decline", is one of the most serious disease of peaches in Georgia. No definite interactions has been revealed in this disease but *Pratylenchus* along with some species of *Pythium* has been found associated with many afflicted trees (Hendrix et al, 1965). Sonto and Holtzman (1970) working on sugarcane in Hawai reported that simultaneous inoculation of *Pratylenchus zeae* and *Pythium graminicola* caused greater reduction in the top and root growth than with either organism alone, while Apt and Koike (1962) found that there was a definite interaction between root-knot nematode *M. incognita* acrita, *and root-rot fungus* Pythium graminicola *Subrum.* on sugarcane, in reducing top growth but not root growth of the plants, when pathogens were present together.

Dave (1975) reported that damage caused by *R. solani* Kunn to soybean in presence of nematodes was more than additive. *R. solani* inhibited nematode population development. However, *H. glycines* was the most suppressed and *Tylenchorhynchus martini* the least. Gracia and Mitchell (1975) studied the interactions by exposing groundnut seedlings in autoclaved soils to predetermined inoculum densities of the pathogens alone or in combinations. No single pathogens (*Pythium myriotylum, F. solani, R. solani and Meloidogyne arenaria)* caused significant damping-off at the density employed. *P. myriotylum* interacted significantly with *F. solani* and *M. arenaria* but not with *R. solani* in causing damping-off.

Garcia and Mitchell, (1975) reported that *Meloidogyne arenaria* (Neal) Chitwood interacted synergistically with *Pythium myriotylum* on peanut causing severity in pod rot and also interact with *Aspergillus flavus* Link to cause increased root disease which was accompanied by reduced nematodes multiplication (Patel et al, 1986).

Anwar and Alam (1998), studied the influence of *M. incognita* infection on incidence of *P. apanidermatum* in tomato cv. Pusa Ruby. They found that sequential inoculation of both

pathogens irrespective of time interval caused the greater reduction in plant growth. The maximum reduction in plant length and weight was recorded when *M. incognita* and *P. aphanidermatum* were inoculated simultaneously.

3. Interaction between Nematodes and Seedling Diseases:

Fungi and nematodes combine to increase root damage in the disorders in early stages of plant growth, which may be classified as seedling disease. it is very difficult to differentiate between root-rot disease from seedling diseases on the basis of symptoms and nature of infection which are quite similar in both the cases. The differences are based on the stage of the plant at the time of infection and disease development. In the seedling diseases, as in root-rot and wilt diseases, nematode-fungus combination bring about more damage to the plants. Under green house condition, the citrus nematode, *Tylenchulus semipenetrans* interact with *F. solani* resulting greater damage of citrus seedlings than caused by either of the pathogen alone (Van Gundy and Tsao, 1963). Damping-off of seedlings in several crops caused by *Pythium spp.*, increased in the presence of nematodes.

Norton (1960) demonstrated that damping-off of cotton caused by *Pythium debaryanum* was much greater in seedlings

inoculated with fungus in combination with root-knot nematode, *M. incognita acrita* than with the fungus alone and suggested that nematode provided additional penetration sites for the fungus. The association of both the nematodes *M. javanica* and *M. hapla* increased the pre-emergence damping off soybean seedlings in the presence of *Rhizoctonia solani* (Taylor and Wyllied, 1959).

Townshend (1984) reported that *M. hapla* appeared to exacerbate the loss of alfalfa seedlings in soil contaminated with *Pythium ultimum*. Similarly, Griffin and Thyr (1988) reported that *M. hapla* is essential for the infection of alfalfa by *F. oxysporum* although this fungus also appeared to suppress the nematode reproduction.

Migratory ectoparasitic nematodes also play an important role in seedling disease complexes with many fungi and cause considerable damage to the plants. The harmful effects were observed on alfalfa by Edmund (1964, 1968) when plants were inoculated with *Pratylenchus peretrans* in the presence of *Trichoderma viridae* and *Fusarium oxysporum*.

Castillo et al., (1977) reported reduced seedling emergence and a seed yield reduction of 28.9% caused by *M. incognita* in

infested field plots. High density level of *M. javanica* may kill mungbean seedlings (Castibog and Castillo, 1975).

Singh (1977) studied the effect of root-knot nematodes, *Meloidogyne incognita* (Kofoid and White 1919) Chitwood 1949, alone and in combination with some *Fusarium* spp. on seedling emergence and growth of cauliflower, brinjal and tomato.

Shepherd-RL (1984) reported the effect of root-knot nematode alone and in combination with fungi on cotton seedling diseases. *Meloidogyne incognita* has been reported to increase the incidence of cotton seedling diseases and fungi that interact synergistically with this nematode include *Fusarium oxysporum* f. sp. *vasinfectum, Rhizoctonia solani* and *Pythium debaryanum.*

Pandy (1984) reported that *M. incognita, P. ultimum* and *R. solani* reduced the growth of sugarbeet seedlings but maximum reduction was seen where plants were inoculated with *M. incognita* followed by *P. ultimum* or *R. solani* or *P. ultimum+R. solani.*

Pastucha (1998) reported that the roots and stem base of soybean seedlings were most frequently infected by *R. solani* and *F. solani* while the plants anthesis were mainly infected by *F. oxysporum* f.sp. glycine.

Roux-A-le and Le-Roux-A (1990) reported the seedling disease of cotton caused by *Rhizoctonia solani* but other fungi including *Fusarium* spp., *Pythium* spp. on *Macrophomina phaseolina* may also be involved.

Effect on Photosynthetic Pigments:

Chlorophylls, the green pigments of plants are the most important pigments responsible for the conversion of light energy into chemical energy and thus active in process of photosynthesis. Chlorophyll a and b are the most abundant pigments in the plants. Nematode and fungi are known to interact with the metabolism of photosynthetic pigments in plants. However, the information about their influences on the photosynthetic pigments and photosynthesis is limited.

Effect of Nematodes on Photosynthetic Pigments:

When nematodes infect roots of the host plant, they rapidly disrupt the physiological process of the host plant in one way or the other. Several workers reported the effect of nematodes on photosynthetic pigments of host plants. Loveys and Bird (1973) reported that *M. javanica* infection caused the reduction in net photosynthetic rate and chlorophyll contents of tomato.

Nematode invasion is also known to bring a change in the concentration of nutrient elements such as Fe, Zn, Mn, K etc. which play an important role for the constituents of plants eg. Fe and Mn in photosynthetic pigments. Change in the concentration of these elements in plants even to a small extent, appear to have a profound impact on host physiology which in turn a major cause in limiting the growth of host plant and cause imbalance in translocation process (Bird and Loveys, 1975, McClure, 1977).

Melakeberhan et al., (1985a) found a significant decrease in the chlorophyll content of frenchbean within two weeks after infection with different inoculum levels of *M. incognita*. In another study Melakeberhan et al., (1986) observed that the chlorophyll content at the end of experiment was markedly lower in the frenchbean plants inoculated with *M. incognita* (single generation) at the bud stage than at the earlier stages. While chlorophyll b content in all plants did not change significantly with increasing inoculum levels, the total chlorophyll (a+b) and chlorophyll a content in all plants was significantly lowered with increasing inoculum level.

Upadhyay and Banerjee, (1986) reported that decrease in the chlorophyll contents of chickpea due to *M. javanica* infection

was because of proportional increase in the concentration of pheophytin. The imbalance in chloroplast pigments may be correlated with the general chlorosis and dieback caused by the infection of nematodes.

Chahal and Chahal, (1987) reported that significant reduction in the chlorophyll contents (chlorophyll a,b) of mungbean leaves caused by increased number of nematodes (*M. incognita)*, which ultimately lead to the reduced production and supply of carbohydrates to nodules for carrying out nitrogen fixation. Quantitative changes in the chlorophyll contents of root-knot infected plants have also been reported by Anver and Alam (1989), and on chickpea (Tiyagi and Alam, 1990; Ahmad and Kumar, 1990). Chandel et al., (1993) observed a higher reduction in the chlorophyll contents of susceptible pigeonpea cultivar when inoculated with *M. incognita*. Vashishth et al., (1994) reported a similar effect of root-knot nematode on the chlorophyll contents of some black gram cultivars.

Effect of Fungi on Photosynthetic Pigments:

Fungi are also associated with legumes and they reduce the photosynthetic pigments of plants. Murukumar and Chawan, (1985) reported that wilt fungus *Fusarium oxysporum* f.sp. *ciceri*

caused a marked reduction in chlorophyll content of chickpea, while as Singh et al, (1986b) found reduced chlorophyll and carotenoid contents in downey mildew (*Pernospora megasperma)* infected leaves of opium.

Agrawal et al, (1982) noted that *Taphrina maculans* Butler infected leaves of turmeric *(Curcumg longa L.)* possessed lower contents of chlorophyll than the healthy ones. Similarly, Sharma and Sharma (1990), noticed marked decrease of chlorophyll content in *Taphrina deformans* infected peach leaves. Srinivasan (1982) reported similar effect on arecanut infected with yellow leaf spot disease.

Prasad et al., (1989) reported reduction in chlorophyll (a, b and total), carotenoids and xanthophylls contents were also recorded in the leaves of coriander infected with *Protomyces macrosporus*. Buonaurio (1991) determined the chlorophyll content of chloroplasts from faba bean leaves infected with *Uromyces viciae* (Pers.) Schroet and observed decreased chlorophyll contents from the beginning of urediospore differentiation to pastule eruption (8-14 days) after inoculation. In addition there was a significant reduction in chlorophyll a/b ratio. Similarly, in onion leaves infected with *Pernospora destruetor*

(Berk.) Casp., a significant gradual loss in the contents of chlorophyll a,b and total chlorophyll was observed with an increase in the infection of foliage (Sugha et al., 1992).

Effect of Nematode-Fungus Disease Complexes on Photosynthetic Pigments:

Only few reports are available on the combined effect of nematode-fungus disease complexes on the chlorophyll contents of plants. Anwar et al., (1997) while studying the interaction between *Meloidogyne incognita* and *Rhizoctonia solani* on soybean observed significant alterations in chlorophyll a and b by simultaneous, sequential and individual inoculations but more damage occurred with simultaneous inoculation than other treatments.

Tiyagi, (1990) while studying the effect of *M. incognita* and *Macrophomina phaseolina* alone and in combination on the chlorophyll content of mungbean observed a significantly higher reduction in chlorphyll content (a,b and total) in concomitant inoculations than in either of them alone. Similar effect in photosynthetic pigments (Chlorophyll and Carotenoid) was also noticed in simultaneous inoculation of *M. incognita* and *R. solani* on the potted frenchbean plants (Shah, 1993).

Effect of Nematode-Fungus Disease Complexes on Root Nodulation:

The infection of nematode and fungi caused reduction in pulse crops. The reduction of root nodulation occurred considerable when pathogen infect individually but in their concomitance the reduction in root nodulation has been found to be much more pronounced.

Anwar et al., (1997) observed greater inhibition of nodulation on soybean with simultaneous inoculation of *R. solani* and *M. incognita*. Nematode inoculation prior and after fungus treatment resulted in only slight to moderate inhibition of nodulation. Hussain et al., (1995) reported significantly higher reduction in the nodulation of pea when three pathogens *M. incognita, R. solani* and pea mosaic virus were inoculated simultaneously then any one of them alone. Mani and Sethi (1987) reported similar results on chickpea by inoculation *M. incognita*, *Fusarium oxysporum* f.sp. *ciceri* and *Fusarium solani* simultaneously. Adverse effects on nodulation was also noted by Tiyagi (1990) on mungbean and Siddiqui and Husain (1991) on chickpea upon the concomitant inoculation of *M. incognita* and

M. phaseolina and by Anwar and Verma (1993) on chickpea in presence of *M. javanica* and *R. solani.*

Kassab and Ali (1996) reported the interaction among *Meloidogyne incognita*, *Rotylenchulus reniformis, Rhizoctonia solani* and *Rhizobium* on cowpea. *M. incognita* alone, slightly promoted plant growth and nodule formation. *R. reniformis* suppressed plant growth and nodule formation. *R. solani* was pathogenic and severely damaged plants and nodular growth and when inoculated in combinations with *M. incognita* it predisposed the plants to gall formation and nematode fecundity.

Materials and Methods

MATERIALS AND METHODS

The different materials to be used and the methods to be employed during the course of proposed experimental programme are generalized as follows:

Selection of Test Plant and Pathogens:

In the proposed plan of work the root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood, 1949, the wilt fungus, *Fusarium oxysporum* Schlechtendahl Freis f. sp. *Glycine* and the root-rot fungus, *Pythium aphanidermatum* (Edson) Fitzp. have been selected as test pathogens for the studies. Soybean (*Glycine max.* var L.) used as test plant during summer season. Respective Rhizobia of this test plant was also used.

Preparation and Sterilization of Soil Mixture:

Sandy loam soil, which is commonly found in Aligarh was collected from fertile field at Aligarh Muslim University Agricultural farm. The soil was then passed through a coarse sieve (1mm pore size) to remove stone particles and debris etc. Organic manure at the rate of 3:1 (soil: organic manure) was thoroughly mixed with the soil and earthen pots (15cm) were filled with this soil-organic manure mixture@1Kg/pot. A little

water was poured in each pot before transferring them to autoclave for sterilization at 20-lb. pressure for one hour. Sterilized pots were allowed to cool down at room temperature before use for experiments.

Raising and Maintenance of Test Plant:

The seeds of test plant, *Glycine max*. var. L. were surface sterilized with 0.1% mercuric chloride for 2 minutes, and thoroughly washed thrice in distilled water and sown in clay pots (15cm diameter), containing steam sterilized soil. After emergence, the seedlings were thinned and only one seedling was allowed to grow in each pot. One week old seedlings at three leaf stage were inoculated with test pathogens, as per the schedule of the experiments.

Preparation of Nematode Inoculum:

Single egg-mass obtained from the root of brinjal *(Solanum melongena)* infected with root-knot nematode, *M. incognita* was collected from brinjal field around Aligarh, was surface sterilized with chlorox (calcium hypochloride) for 5 minutes and then washed thrice in sterilized distilled water. The egg-mass was then allowed to hatch in sterilized distilled water at 27°C, the larvae thus obtained were used for further inoculation of egg plants to

maintain a regular supply of pure inoculum for experimental work.

Culture of root-knot nematode, *M. incognita* was maintained on brinjal in concrete microplots. Egg masses were handpicked from heavily infected brinjal roots with the help of sterilized forcep from the previously maintained pure culture of *M. incognita* on brinjal. The egg masses were washed with sterilized water and placed on a small coarse sieve (1 mm pore size) fitted with moist tissue paper. Later the sieve was placed in 10 cm. diameter petridish containing water just touching its lower portion. A series of such assemblies were kept to get required number of second stage juveniles (J_2) for inoculation. The second stage juveniles (J_2) were hatched out after 24 hours at 27°C in an incubator (Stemerding, 1963). After every 24 hours the nematode suspension (J_2) was collected in beakers from petridishes. Fresh water was added to petridishes after with drawing the nematode suspension every time. This process was repeated upto 5-7 days. These second stage juveniles served as the inoculum of root-knot nematode. The second stage juveniles of *Meloidogyne incognita* were counted with the help of counting dish under the stereoscopic microscope (Southey, 1986). An average of 3 to 5 counts were made to determine the

approximate population of nematode juveniles in the suspension. Calculations were made for the total suspension for nematode larvae to be used for inoculation.

Isolation of Fungus from Infected Roots:

Soybean (*G. max*) plants showing distinct galls and exhibiting root-rot and wilt symptoms were collected in polythene bags from infected fields. Serial washing technique (Harley and Waid, 1955) was employed to isolate fungus from infected roots. Roots were transferred to a sterilized dish containing sterile distilled water and gently freed of soil particles. The process was repeated till all the soil particles were removed. The roots were cut into small pieces approximately 5mm and transferred to petridishes containing 0.1% mercuric chloride solution. After one minute, root pieces were washed at least three times in distilled water and dried on filter paper. Five of these root pieces were then placed in each of 10 petriplates containing potato dextrose agar (PDA) (Peeled potatoes, 200 gm; Dextrose, 20gm; Agar-Agar, 20gm and distilled water, 1000ml).

Petriplates were incubated at 28±2°C for 10 days. The fungi which developed on root segments were examined and identified. After confirmation of fungus identify, the pure cultures

of *Fusarium oxysporum* and *Pythium aphanidermatum* were prepared.

Raising and Maintenance of Fungus Culture:

For obtaining sufficient inoculum the two fungi were later cultured on "Richard" liquid medium (Potassium nitrate 10g, Potassium dihydrogen phosphate 5.0g, Magnesium sulpahte 2.5g, Ferric chloride 0.02g, Sucrose 50.0g and distilled water 1000 ml).

The medium was prepared and filtered through muslin cloth and then sterilized in an autoclave at 15-lb pressure for 15 minutes in 250ml Erlenmeyer conical flasks each containing 100ml of liquid medium. Later the fungus was inoculated in sterilized flasks with the help of sterilized inoculation needle, in an aseptic chamber. Inoculated flasks were then incubated at 28±2°C for about 15 days to allow sufficient growth of the fungus.

Pure cultures of both the fungi were continuously maintained on PDA by reinoculation of the fungus after every 15 days.

After incubating the flasks for about 15 days the required medium was filtered through Whatman's filter paper No.1 The

mycelial mat was washed in distilled water and gently pressed between sterile blotting sheets to remove excess amount of liquid. The inoculum was prepared by mixing 10gm fungal mycelium in 100ml of distilled water and blending it for 30 seconds in a mixer (Stemerding, 1964). In this way each 10ml of this homogenate contained 1gm of fungal mycelium.

Inoculation Techniques:

One week old seedlings (three leaf stage) of Soybean (*Glycine max*. var. L.) were inoculated with 1000 second stage juveniles (J_2) of *Meloidogyne incognita*, 1gm mycelium of *Fusarium oxysporum* and *Pythium aphanidermatum* throughout the course of these investigations, unless stated outherwise. Feeder roots of seedlings were exposed just before inoculation, holes of 5-7 cm depth around the plants within a radius of 2cm from the plants were made in which a counted number of nematode larvae (second stage infective juveniles) and fungal suspension were transferred with the help of sterilized pipette. The holes were then plugged with sterilized soil. Both individual and combined inoculation of the pathogens were done depending upon the experiment. Throughout these studies each treatment was replicated three times and uninoculated plants were kept as

control. Regular watering was done to maintain the soil moisture. Experiments were terminated after 90 days of inoculation.

Hatching Test:

For hatching experiment, 5 egg-masses of same size were transferred to each petridish containing 10ml of different dilutions of culture filtrates of each fungus. Petri dishes were kept at 25°C temperature. The hatched larvae were counted after 48 hours of incubation. The egg masses placed in distilled water served as control. Each treatment was replicated thrice.

Mortality Test:

To study the effect of culture filtrates on larvae of *Meloidogyne incognita*, the two fungi grown for 15 days in 250 ml of liquid medium were filtered through Whatman filter paper No.1. Filtrate, thus obtained, have been arbitrarily termed as 'S' solution. Different dilutions were prepared in sterilized distilled water from the Standard solution viz. S/2, S/10, S/100, S/1000, S/10000. To 10ml of each solution contained in Syracuse watch glasses, 100 hand-picked larvae of *M. incognita* were transferred. The same number of larvae placed in distilled water served as control. Later, the watch glasses were kept in an incubator running at 25°C temperature. There were three

replicates for each treatment. Counts of larvae that became immobilised in each dilution were made after 24, 48 and 72 hours.

RECORDING OF DATA:

Parameters Used:

The plants were uprooted after 90 days of inoculation and their roots were gently washed off the soil, taking utmost care to avoid losses and injury to roots during the entire operation. The excess of water was removed by putting the plant parts between the folds of the blotting sheets, for some time before taking their fresh weights. For measuring length and weight the plants were cut with sharp knife just above the base of the root emergence zone. The length of the shoots and roots was recorded in centimeters from the cut end to the top of the first leaf and to the longest root respectively. The weight was recorded in grams. For dry weight, the roots and shots were kept in bamboo envelopes for drying in an oven at 60°C for 2-3 days. Reduction in dry plant weight (root+shoot) was calculated in terms of percent reduction for the interpretation of the results.

Root Nodule Estimation:

Nodulation was estimated by counting the number of nodules per root system and percentage of nodule reduction over control was calculated.

Root-knot Estimation:

The root-knot nematode galls were estimated by counting the number of galls per root system. The degree of root infection caused by root-knot nematode was assessed according to the rating scale of Taylor and Sasser (1978) for the presence of root galls as under

1 = No galls,

2 = 1-10 galls,

3 = 11-30 galls,

4 = 31-100 galls and

5 = 101 and above galls.

Wilt Estimation:

After inoculation, the pots were kept in benches of net house in complete randomized block design (CRBD). Plants were observed regularly for appearance of wilt symptoms. The intensity of wilt symptoms was determined after 90 days of

inoculation. The wilt index rated on 0-4 scale as given below (Sidhu and Webster, 1987).

0 = No symptoms,

1 = Light symptoms,

2 = Moderate symptoms,

3 = Heavy symptoms and

4 = Severe symptoms (dead)

Root-rot Estimation:

The rating scale for root-rot estimation was observed as follows:

1 = Less than 10% root-rot,

2 = 11-25% root-rot,

3 = 26-50% root-rot,

4 = 51-75% root-rot and

5 = 75-100% root-rot (severe root-rot)

Statistical Analysis:

The data obtained were analysed statistically and significance of variance was calculated at P (0.05) and P (0.01) levels.

Results

RESULTS

1. Effect of different concentrations of culture filtrates of *Fusarium oxysporum and Pythium aphanidermatum* on the hatching of IInd stage larvae of *Meloidogyne incognita:*

The effect of different concentrations of culture filtrates of the two fungi on cumulative hatch of *M. incognita* is given in Table 1.a. The different concentrations of culture filtrates of the two fungi significantly reduced the hatching of larvae of *M. incognita*, except S/100 where hatching was stimulated. There had been a direct relationship between suppression of hatching and the dilution of culture filtrate, and it remains significantly low upto S/10 dilution of the two fungi. However, the extracts of the two fungi differed adversely affecting the hatching. The number of larvae hatched was more in all the dilutions of culture filtrate of *P. aphanidermatum* than the *F. oxysporum*.

2. Effect of different concentrations of culture filtrates of *F. oxysporum and P. aphanidermatum* on the mortality of IInd stage larvae *M. incognita:*

It is evident from Table 1.b that S and S/2 dilution of culture filtrate of *F. oxysporum* brought about 64 and 50 percent mortality after 24 hours of incubation, however the culture filtrate

Table-1.a: Effect of different concentrations of culture filtrates of *Fusarium oxysporum* and *Pythium aphanidermatum* on the hatching of IInd stage larvae of *Meloidogyne incognita*.

Culture filtrates	Concentrations							
	S	**S/2**	**S/10**	**S/100**	**S/1000**	**D.W.**	**CD [P(0.05)]**	**CD [P(0.01)]**
Fusarium oxysporum	19 (96.89)	31 (94.92)	93 (84.77)	253 (58.59)	407 (33.38)	611	18.14	25.79
Pythium aphanidermatum	30 (95.09)	63 (89.69)	149 (75.56)	315 (48.44)	480 (21.44)	611	14.24	20.24

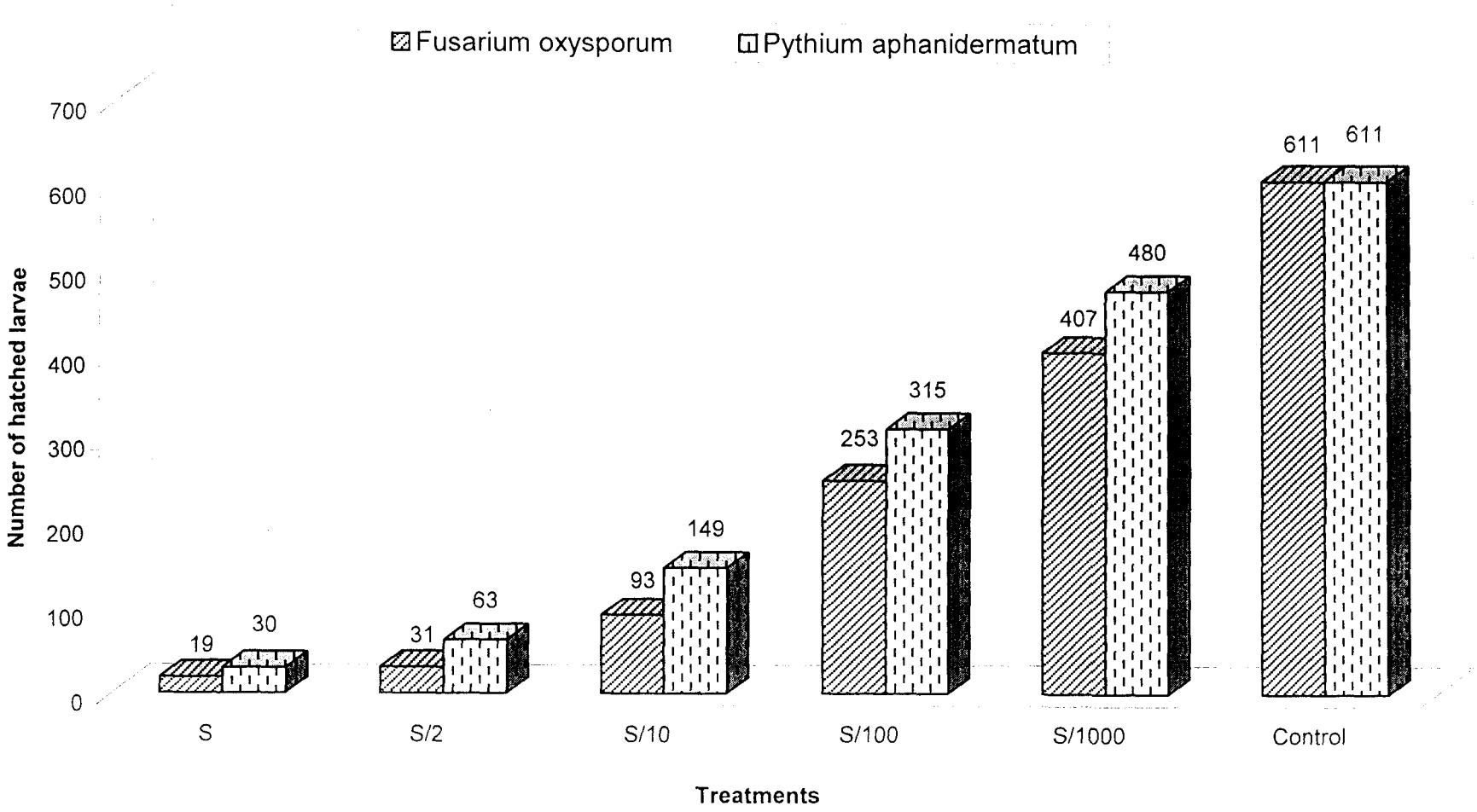

Fig.1.a: Effect of different concentrations of culture filtrates of *Fusarium oxysporum* and *Pythium aphanidermatum* on the hatching of IInd stage larvae of *Meloidogyne incognita*.

Table-1.b: Effect of different concentrations of culture filtrates of *Fusarium oxysporum* and *Pythium aphanidermatum* on the mortality of IInd stage larvae of *Meloidogyne incognita*.

Culture filtrates	Concentrations								
	Exposure time	S	S/2	S/10	S/100	S/1000	D.W.	CD [P(0.05)]	CD [P(0.01)]
F. oxysporum	24h.	64	50	10	0	0	0	3.02	4.31
	48h.	97	59	16	11	4	0	3.44	4.89
	72h.	100	90	23	15	8	0	3.72	5.28
P. aphanidermatum	24h.	55	42	7	0	0	0	3.29	4.68
	48h.	83	52	12	7	2	0	2.28	3.24
	72h.	93	83	17	10	6	0	2.55	3.63

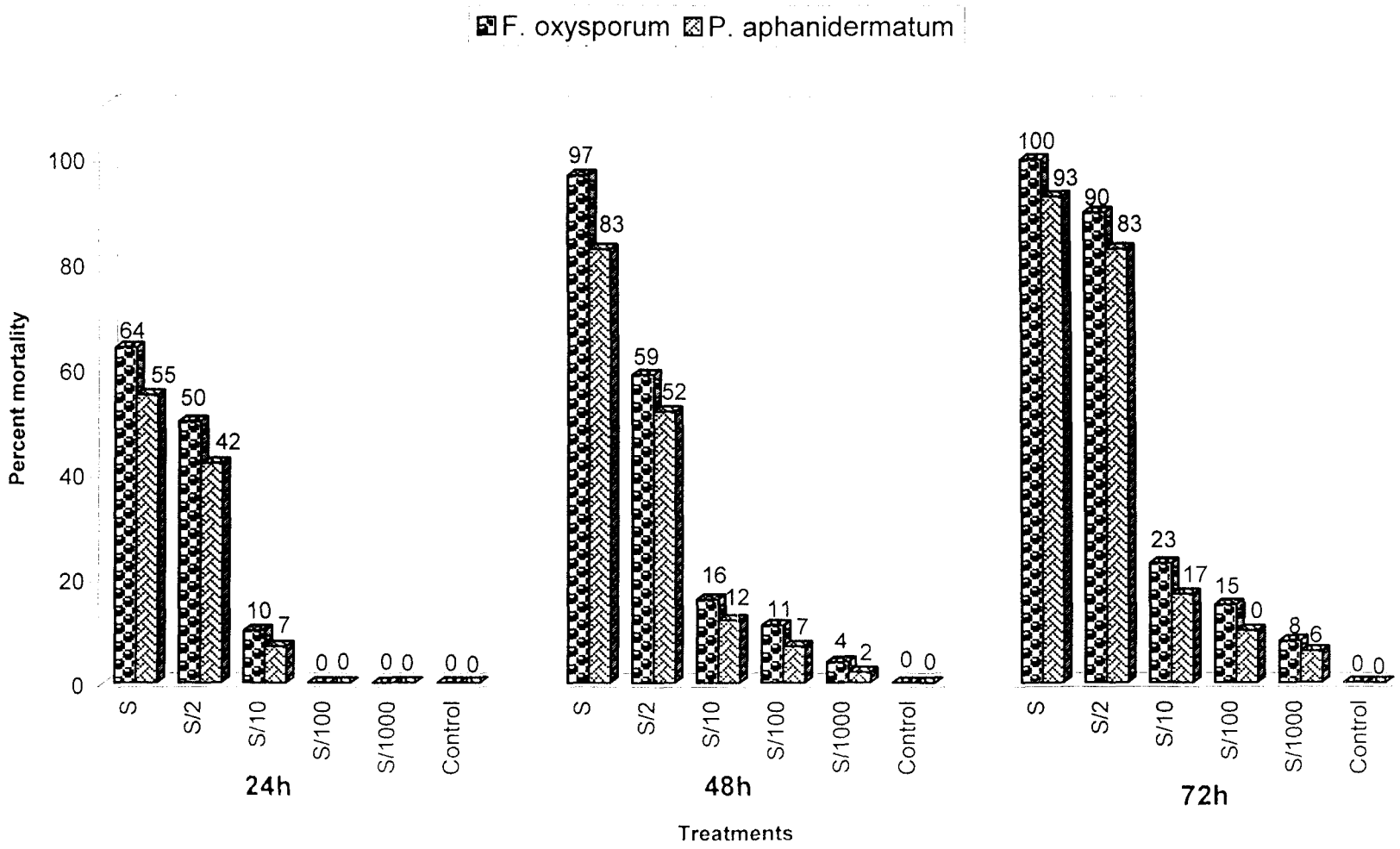

Fig. 1.b: Effect of different concentrations of culture filtrates of *Fusarium oxysporum* and *Pythium aphanidermatum* on the mortality of IInd stage larvae of *M. incognita.*

of *P. aphanidermatum* was less lethal as there was 55 and 42 percent killing for the corresponding dilutions. Exposure of larvae in S concentration of *F. oxysporum* for 72 hours brought about 100 percent mortality while in S concentration of *P. aphanidermatum* for 72 hours mortality was 93 percent. Dilutions beyond S/10 had no effect on mortality of larvae after 24 hours and had only little even when they were exposed for 72 hours. The percentage mortality was high in higher concentrations of culture filtrates of both fungi. The culture filtrate of *F. oxysporum* was more toxic to the larvae of *M. incognita* than *P. aphanidermatum*.

3. Effect of different inoculum levels of *M. incognita* on plant growth, number of pods, chlorophyll contents, nematode development, nodulation and gall formation:

It is evident from the data given in Table 2.a & b that an increase in nematode inoculum levels decreased the plant growth of *G. max*. The root length of plant was recorded by 38.20, 34.01, 28.10 and 27.00cm as compared to 41.10cm in uninoculated control and shoot length of plants was 42.00, 38.50, 32.03 and 31.50cm as compared to 44.50cm in uninoculated control in 250,500, 1000 and 2000 juveniles of *M. incognita*

Table-2.a: Effect of different inoculum levels of *Meloidogyne incognita* on plant growth and number of pods on *Glycine max* var. L.

Treatments	Length (cm)				Fresh wt. (g)				Dry wt. (g)				No. of Pods	% Decrease
	Shoot	Root	Total	% Decrease	Shoot	Root	Total	% Decrease	Shoot	Root	Total	% Decrease		
Control (uninoculated)	44.50	41.10	85.60	-	23.00	12.00	35.0	-	8.00	1.801	9.80	-	25.33	-
M. incognita														
250	42.00	38.20	80.20	6.31	20.03	9.20	29.23	16.49	6.82	1.203	8.02	18.16	21.67	14.45
500	38.50	34.01	72.51	15.29	18.50	8.54	27.04	22.74	6.21	0.954	7.16	26.94	19.33	23.69
1000	32.03	28.10	60.13	29.75	16.00	7.31	23.31	33.40	5.35	0.710	6.06	38.16	17.33	31.58
2000	31.50	27.00	58.50	31.66	15.80	7.05	22.85	34.71	5.30	0.654	5.95	39.29	16.00	36.83
CD [P(0.05)]			3.83				2.11				0.94		1.29	
CD [P(0.01)]			5.57				3.07				1.36		1.87	

Table-2.b: Effect of different inoculum levels of *Meloidogyne incognita* on chlorophyll contents, nematode development, nodulation and gall formation.

Treatments	Chl. (a) 'mg/g'	% decrease (a)	Chl. (b) 'mg/g'	% decrease (b)	Total Chl. (a+b) 'mg/g'	% decrease (a+b)	Nematode population			Nematode multiplication Rf=pf/pi	Nodule/root system		No. of galls/root system
							Soil	Tissue	Total		Number	% decrease	
Control (uninoculated) M. incognita	1.200	-	1.501	-	2.701	-	-	-	-	-	75.33	-	-
250	1.100	8.33	1.300	13.39	2.400	11.14	12528.7	109.3	12638.0	50.55	62.67	16.81	78
500	0.890	25.83	1.002	33.24	1.892	29.95	15207.6	127.4	15335.0	30.67	59.33	21.24	97
1000	0.790	34.17	0.920	38.71	1.710	36.69	19993.4	141.6	20135.0	20.13	54.67	27.43	123
2000	0.710	40.83	0.860	42.70	1.570	41.87	29609.8	160.2	29770.0	14.88	47.33	37.17	144
CD[P(0.05)]	0.009		0.015		0.024					1.31	2.92		22.78
CD[P(0.05)]	0.013		0.022		0.035					1.91	4.62		33.27

inoculated plants respectively. Reduction in total plant length was 6.31, 15.29, 29.75 and 31.66% over control in above given treatments respectively.

The fresh weight of root was recorded by 9.20, 8.54, 7.31 and 7.05g as compared to 12.00g in uninoculated control and fresh weight of shoot was 20.03, 18.50, 16.00 and 15.80g as compared to 23.00g in uninoculated control in 250, 500, 1000 and 2000 juveniles of *M. incognita* inoculated plants respectively. Reduction in total plant fresh weight was 16.49, 22.74, 33.40 and 34.71% over control in above given treatments.

The dry weight of root was recorded by 1.203, 0.954, 0.710 and 0.654g as compared to 1.801g in uninoculated control and dry weight of shoot was recorded by 6.82, 6.21, 5.35 and 5.30g as compared to 8.00g in uninoculated control in 250, 500, 1000 and 2000 juveniles of *M. incognita* inoculated plants respectively. Reduction in total plant dry weight was 18.16, 26.94, 38.16 and 39.29% over control in above given treatment respectively.

Total number of pods per plant were recorded by 21.67, 19.33, 17.33 and 16.00 as compared to 25.33 in uninoculated control in 250, 500, 1000 and 2000 juveniles of *M. incognita*

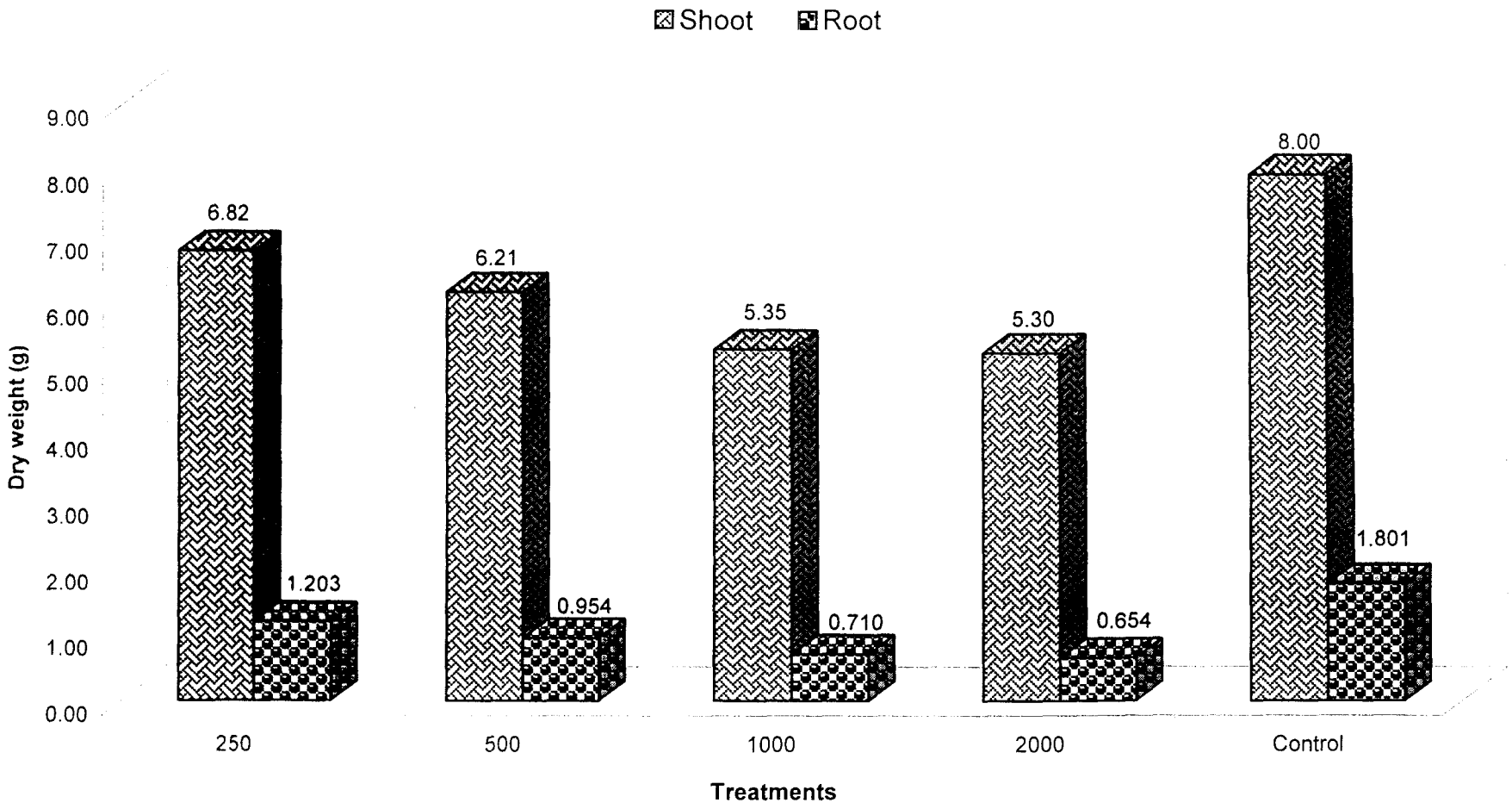

Fig. 2.a: Effect of different inoculum levels of *Meloidogyne incognita* on dry weight of shoot and root.

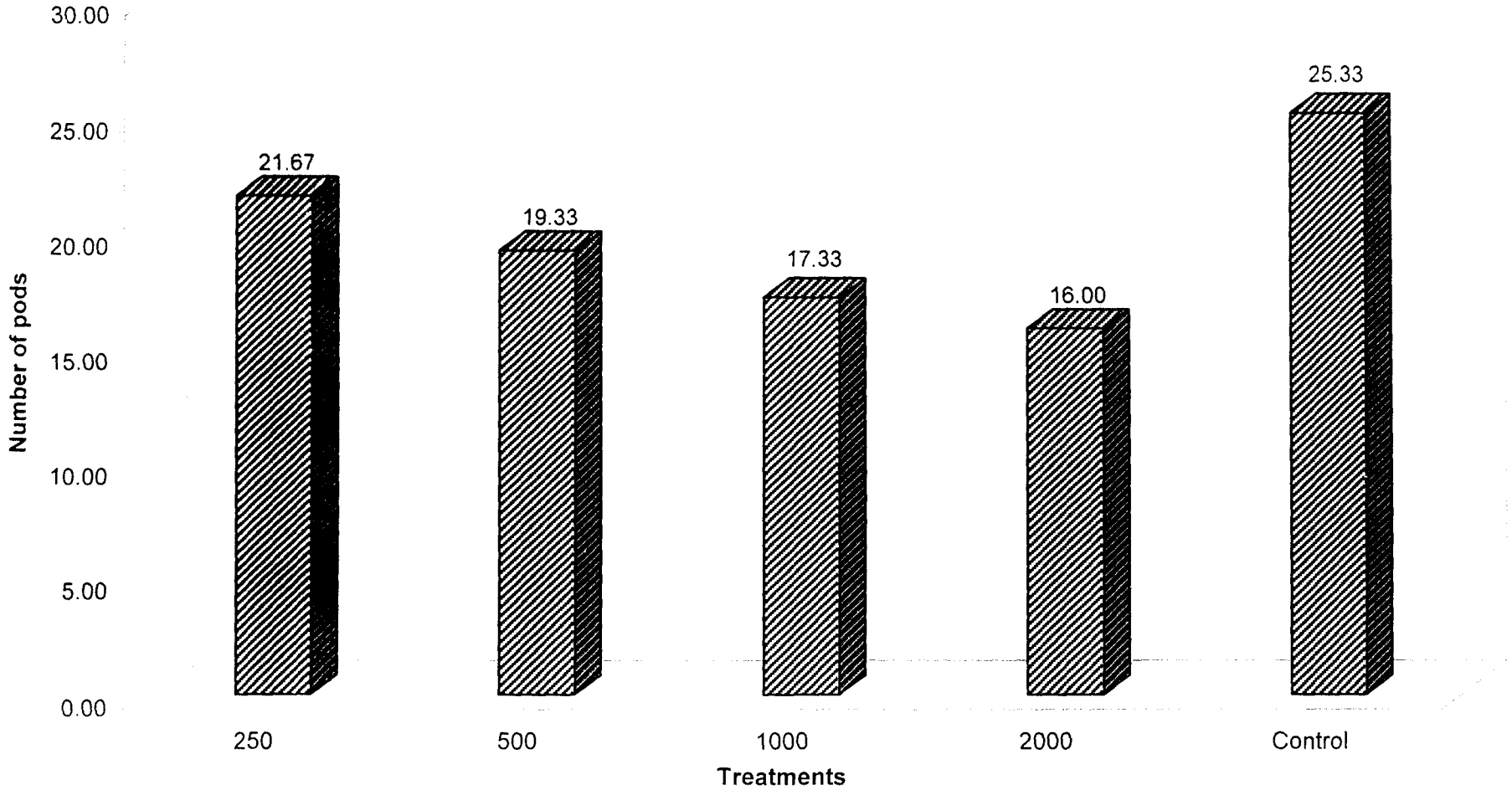

Fig. 2.a: Effect of different inoculum levels of *Meloidogyne incognita* on number of pods.

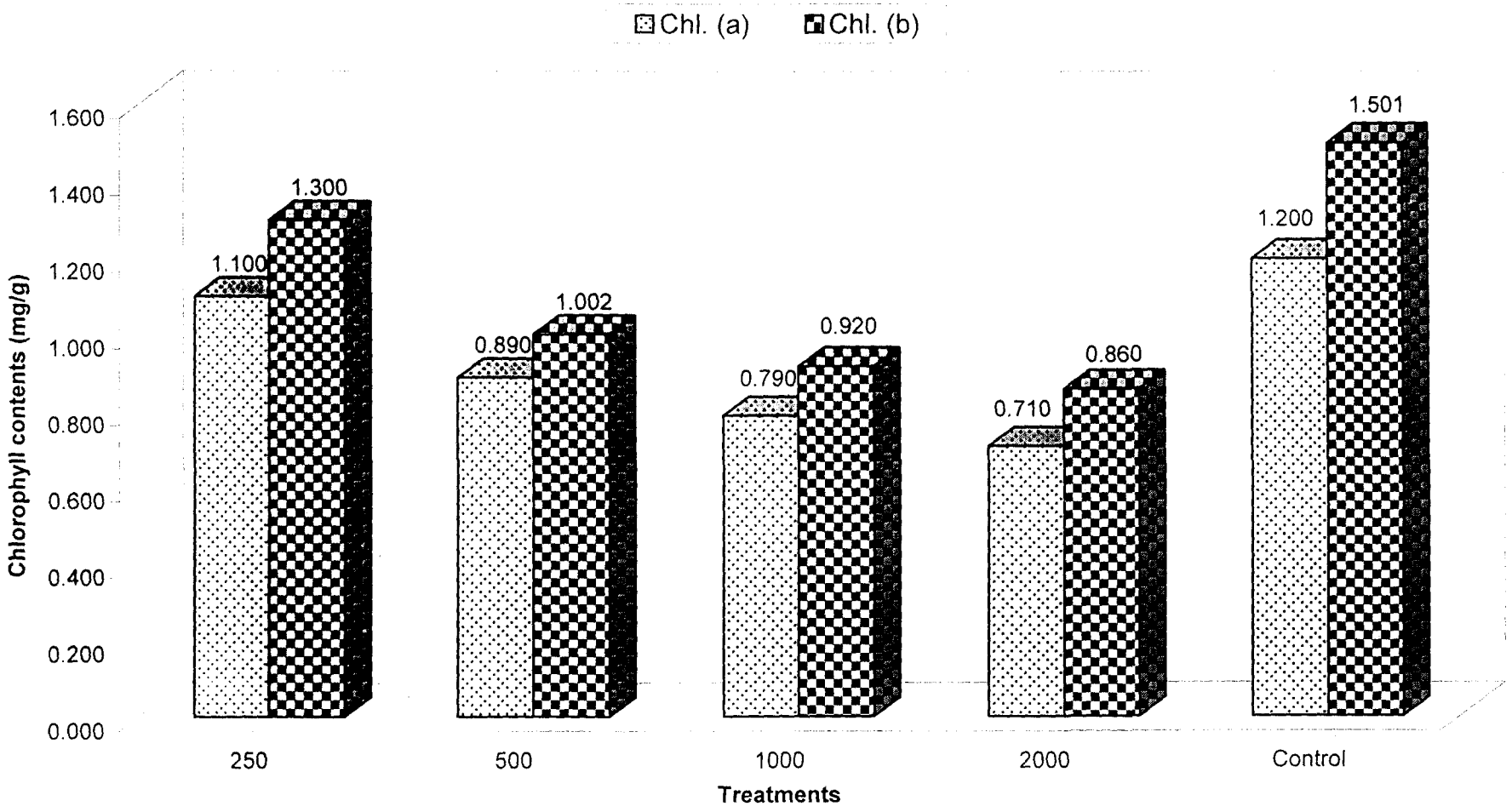

Fig. 2.b: Effect of different inoculum levels of *Meloidogyne incognita* on chlorophyll contents.

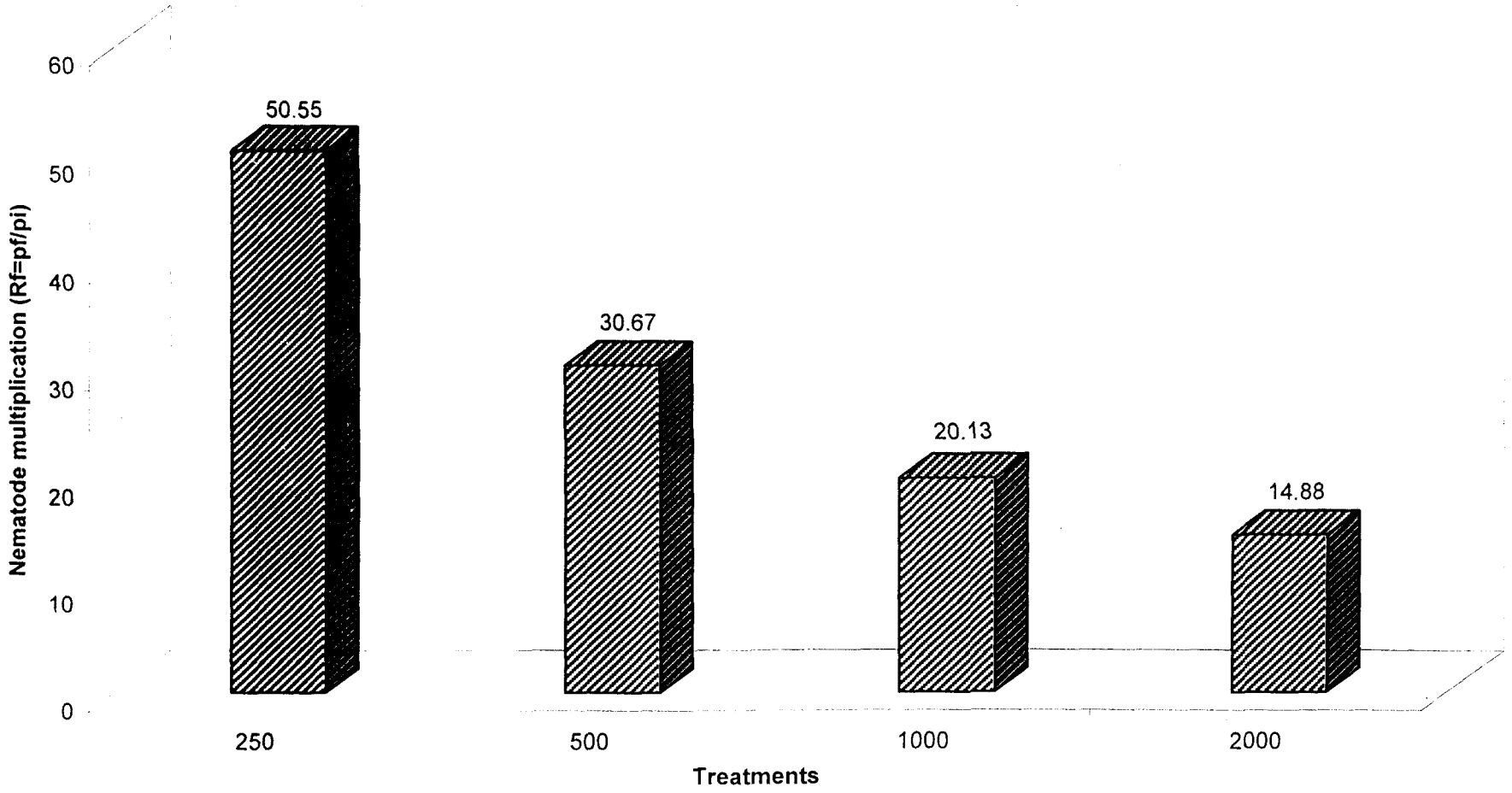

Fig. 2.b: Effect of different inoculum levles of *Meloidogyne incognita* on nematode multiplication.

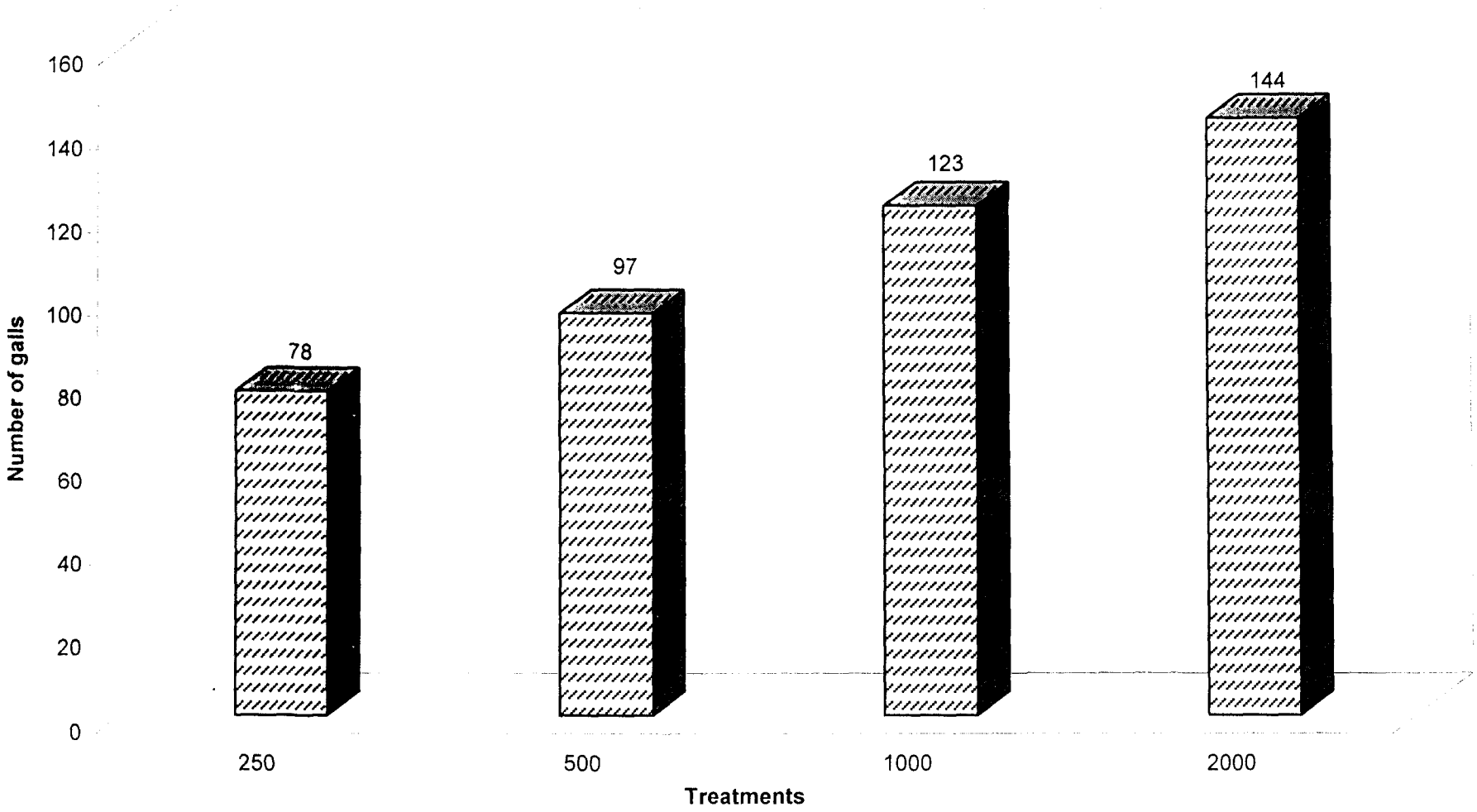

Fig. 2.b: Effect of different inoculum levels of *Meloidogyne incognita* on gall formation.

inoculated plants respectively and reduction in number of pods per plant was recorded by 14.45, 23.69, 31.58 and 36.83% over control in above given treatments respectively.

Chlorophyll 'a' was recorded by 1.100, 0.890, 0.790 and 0.710 mg/g as compared to 1.200 mg/g in uninoculated control in 250, 500, 1000 and 2000 juveniles of *M. incognita* inoculated respectively. Decrease in chlorophyll 'a' was 8.33, 25.83, 34.17 and 40.83% over control in above given treatments respectively.

Chlorophyll 'b' was recorded by 1.300, 1.002, 0.920 and 0.860 mg/g as compared to 1.501 mg/g in uninoculated control in 250, 500, 1000 and 2000 juveniles of *M. incognita* inoculated plants respectively. Decrease in chlorophyll 'b' was 13.39, 33.24, 38.71 and 42.70% over control in above given treatments respectively.

Total chlorophyll (a+b) was recorded by 2.400, 1.892, 1.710 and 1.570 mg/g as compared to 2.701 mg/g in uninoculated control in 250, 500, 1000 and 2000 juveinels of *M. incognita* inoculated plants respectively. Reduction in total chlorophyll (a+b) was 11.14, 29.95, 36.69 and 41.87% over control in above given treatment respectively.

Total nematode population was recorded by 12638, 15335, 20135 and 29770 when plants were inoculated with 250, 500, 1000 and 2000 juveniles of *M. inconita* respectively. The rate of nematode multiplication was 50.55, 30.67, 20.13 and 14.88 in above given treatments, respectively.

Total number of nodules per root system were recorded by 62.67, 59.33, 54.67 and 47.33 as compared to 75.33 in uninoculated control in treatments 250,500, 1000 and 2000 juveniles of *M. incongita* respectively. Reduction in nodules per root system was recorded by 16.81, 21.24, 27.43 and 37.17% over control in given treatments respectively.

Total number of root galls per root system were recorded by 78, 97, 123 and 144 in 250, 500, 1000 and 2000 juveniles of *M. incognita* inoculated plants respectively.

4. Effect of different inoculum levels of *P. aphanidermatum* on plant growth, number of pods, chlorophyll contents, nodulation and root-rot development:

It is evident from data presented in Table 3.a & b that an increase in fungus *P. aphanidermatum* inoculum decreased the plant growth of *G. max* to a varying degree. The root length of

Table-3.a: Effect of different inoculum levels of *Pythium aphanidermatum* on plant growth and number of pods on *Glycine max* var. L.

Treatments	Length (cm)				Fresh wt. (g)				Dry wt. (g)				No. of Pods	% Decrease
	Shoot	Root	Total	% Decrease	Shoot	Root	Total	% Decrease	Shoot	Root	Total	% Decrease		
Control (uninoculated) P. aphanidermatum	44.50	41.10	85.60	-	23.00	12.00	35.0	-	8.00	1.801	9.80	-	25.33	-
0.25(g)	40.50	36.01	76.51	10.62	19.00	8.80	27.80	20.57	6.00	1.102	7.10	27.55	20.67	18.40
0.50	36.10	32.30	68.40	20.09	17.54	8.03	25.57	27.11	5.52	0.804	6.32	35.51	18.67	26.29
1.00	30.08	25.10	55.18	35.54	15.11	6.91	22.02	37.54	4.71	0.553	5.26	46.33	15.67	38.14
2.00	28.01	20.39	48.40	43.46	12.93	6.07	19.00	45.71	4.20	0.501	4.70	52.04	12.67	49.98
CD [P(0.05)]			4.44				2.90				0.95		1.14	
CD [P(0.01)]			6.46				3.62				1.38		1.66	

Table-3.b: Effect of different inoculum levels of *Pythium aphanidermatum* on chlorophyll contents, nodulation and root-rot development.

Treatments	Chl.(a) 'mg/g'	% decrease	Chl.(b) 'mg/g'	% decrease	Total Chl. (a+b) 'mg/g'	% decrease	Nodule/root system		Root-rot index
							Number	% decrease	
Control (uninoculated) P. aphanidermatum	1.200	-	1.501	-	2.701	-	75.33	-	-
0.25(g)	1.075	10.42	1.210	19.39	2.285	15.40	58.00	23.01	1.00
0.50	0.800	33.33	0.900	40.04	1.700	37.06	53.33	29.20	1.30
1.00	0.650	45.83	0.750	50.03	1.400	48.17	48.33	35.84	2.00
2.00	0.570	52.50	0.690	54.70	1.260	53.35	42.33	43.81	3.00
CD [P(0.05)]	0.162		0.024		0.040		3.59		0.74
CD [P(0.01)]	0.024		0.035		0.058		5.22		1.07

plant was recorded by 36.01, 32.30, 25.10 and 20.39cm as compared to 41.10cm in uninoculated control and shoot length of plant was recorded by 40.50, 36.10, 30.08 and 28.01cm as compared to 44.50cm in uninoculated control in 0.25, 0.50, 1.00 and 2.00g mycelium of *P. aphanidermatum* inoculated plants respectively. Reduction in total plant length was recorded by 10.62, 20.09, 35.54 and 43.46% over control in above given treatments respectively.

The fresh weight of root was recorded by 8.80, 8.03, 6.91 and 6.07g as compared to 12.00g in uninoculated control and fresh weight of shoot was 19.00, 17.54, 15.11 and 12.93 g as compared to 23.00g in uninoculated control in 0.25, 0.50, 1.00 and 2.00g mycelium of *P. aphanidermatum* inoculated plants respectively. Reduction in total plant weight was recorded by 20.57, 27.11, 37.54 and 45.71% over control in above given treatments respectively.

The dry weight of root was recorded by 1.102, 0.804, 0.553 and 0.501g as compared to 1.801g in uninoculated control and dry weight of shoot was 6.00, 5.52, 4.71 and 4.20g as compared to 8.00g in uninoculated control in 0.25, 0.50 1.00 and 2.00g mycelium of *P. aphanidermatum* inoculated plants

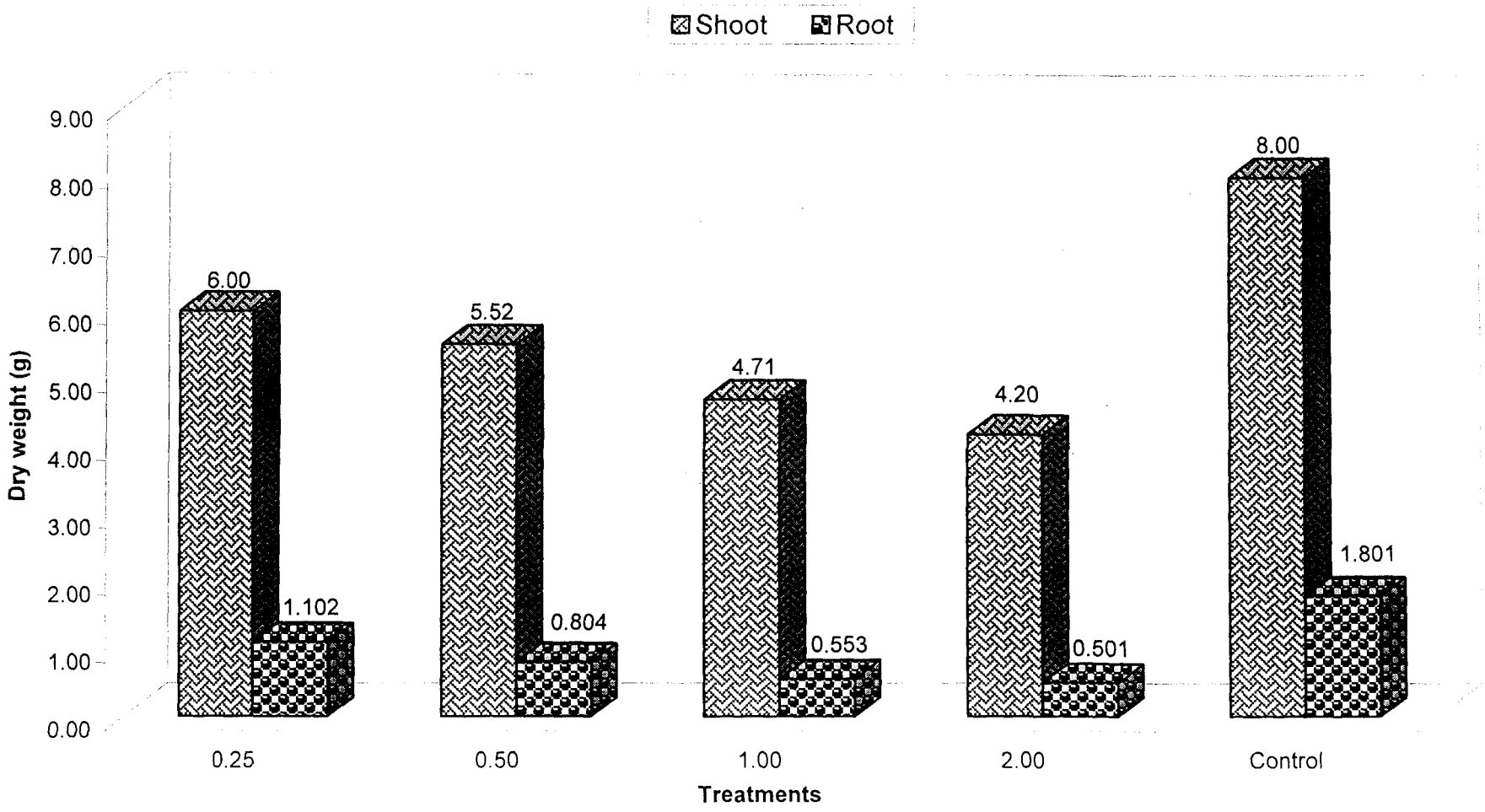

Fig. 3.a: Effect of different inoculum levels of *Pythium aphanidermatum* on dry weight of shoot and root.

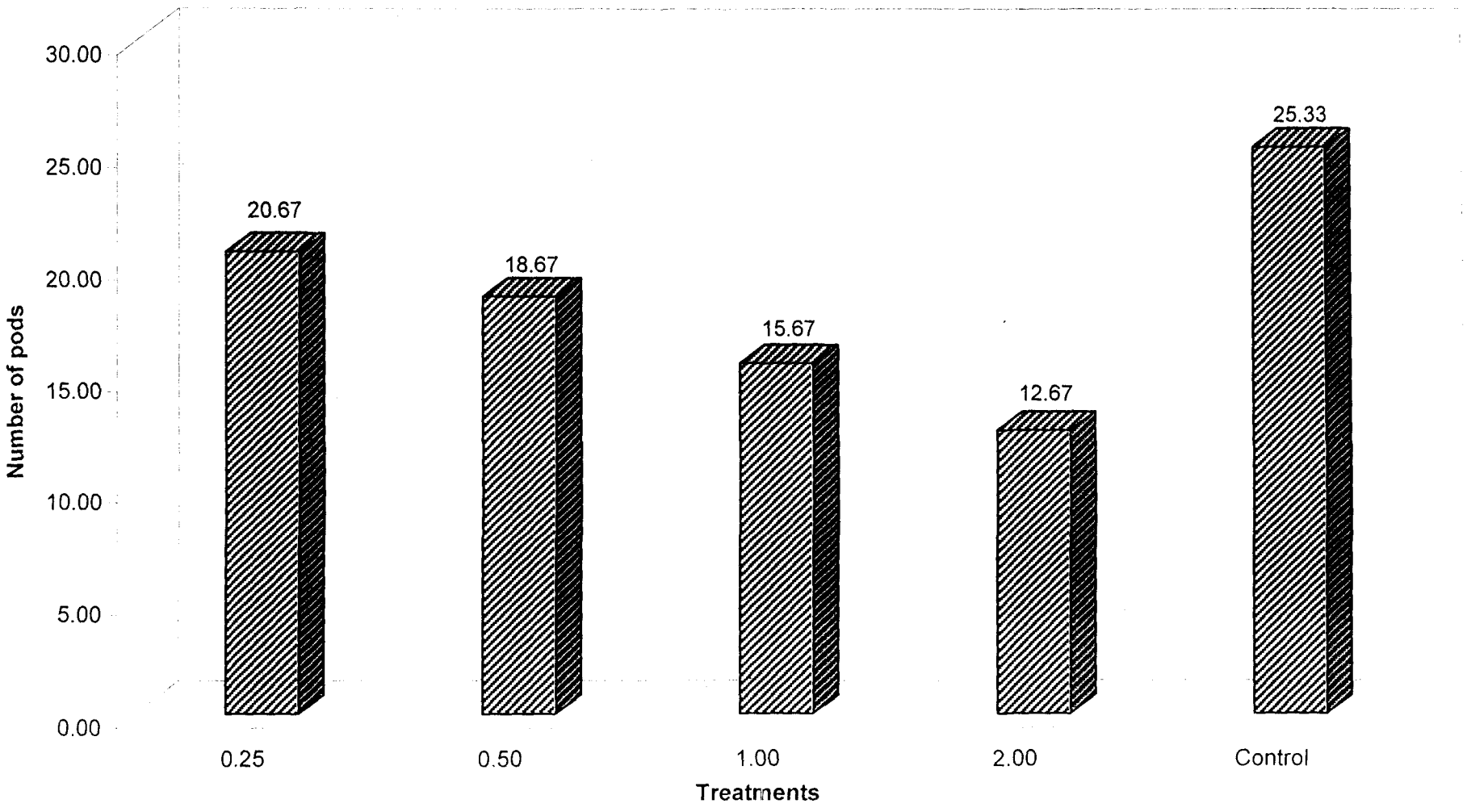

Fig. 3.a: Effect of different inoculum levels of *Pythium aphanidermatum* on number of pods.

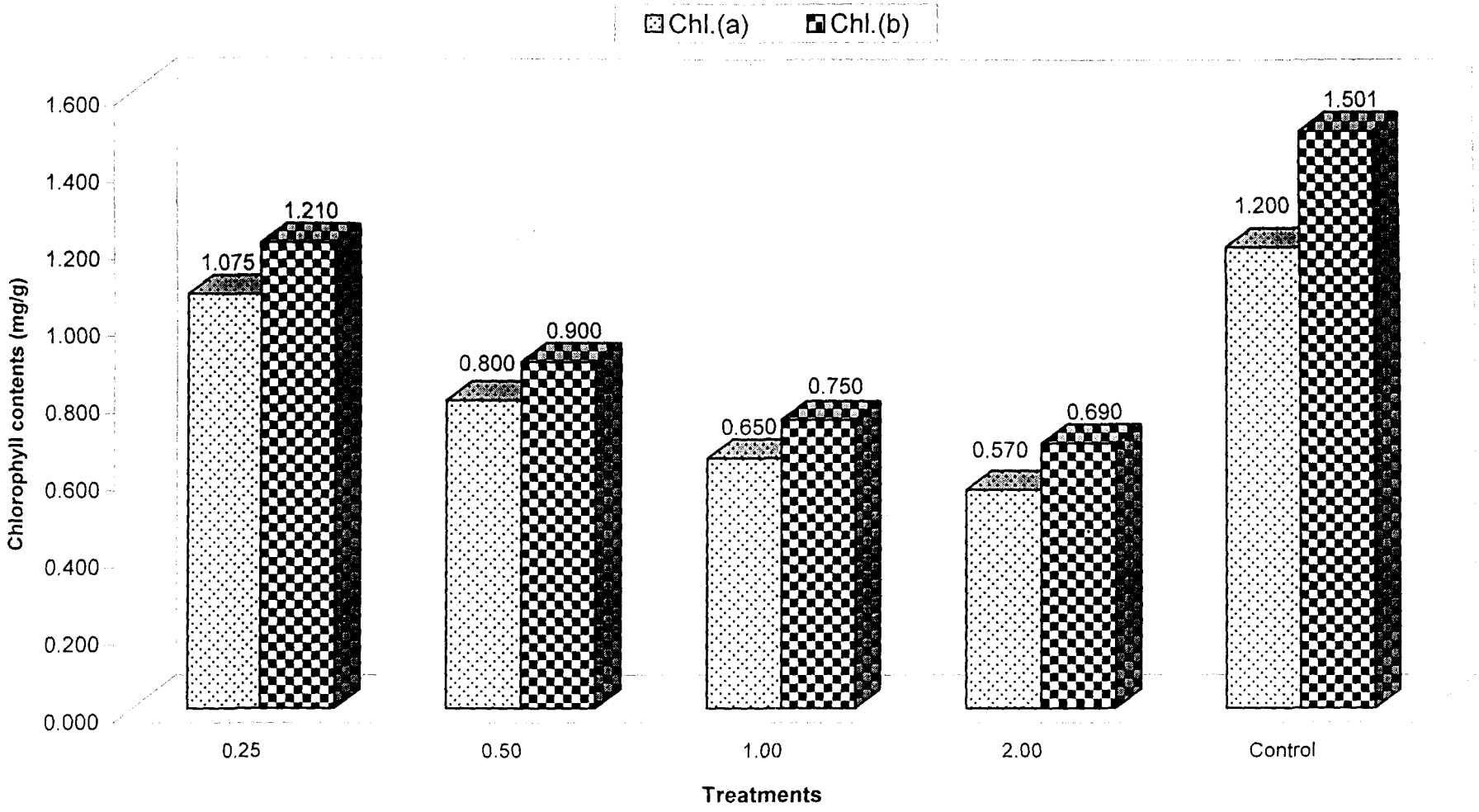

Fig. 3.b: Effect of different inoculum levels of *Pythium aphanidermatum* on chlorophyll contents.

respectively. Reduction in total plant dry weight was recorded by 27.55, 35.51, 46.33 and 52.04% over control in above given treatments respectively.

Total number of pods per plant were 20.67, 18.67, 15.67 and 12.67 as compared to 25.33 in uninoculated control in 0.25, 0.50, 1.00 and 2.00g mycelium of *P. aphanidermatum* inoculated plants respectively. Reduction in total number of pods was 18.40, 26.29, 38.14 and 49.98% over control in above given treatments respectively.

Chlorophyll 'a' was recorded by 1.075, 0.800, 0.650 and 0.570 mg/g as compared to 1.200 mg/g in uninoculated control in 0.25, 0.50, 1.00 and 2.00g mycelium of *P. aphanidermatum* inoculated plants respectively. Decrease in chlorophyll 'a' was 10.42, 33.33, 45.83, and 52.50% over control in above given treatments respectively.

Chlorophyll 'b' was recorded by 1.210, 0.900, 0.750 and 0.690 mg/g in uninoculated control in 0.25, 0.50, 1.00, 2.00 g mycelium of *P. aphanidermatum* inoculated plants respectively. Decrease in chlorophyll 'b' was recorded by 19.39, 40.04, 50.03 and 54.70% over control in above given treatments respectively.

Total chlorophyll (a+b) was recorded by 2.285, 1.700, 1.400 and 1.260 mg/g as compared to 2.701 mg/g in uninoculated control in 0.25, 0.50, 1.00 and 2.00 g mycelium of *P. aphanidermatum* inoculated plants respectively. Reduction in total chlorophyll (a+b) was recorded by 15.40, 37.06, 48.17 and 53.35% over control in above given treatments respectively.

Total number of nodules per root system were recorded by 58.33, 53.67, 48.67 and 42.33 as compared to 75.33 in uninoculated control in treatments 0.25, 0.50, 1.00 and 2.00g mycelium of *P. aphanidermatum* respectively. Reduction in nodules per root system was recorded by 22.51, 28.75, 35.39 and 43.81% over control in respectively.

Total root-rot index was observed 1.00, 1.30, 2.00 and 3.00 in 0.25, 0.50, 1.00 and 2.00g mycelium of *Pythium aphanidermatum* inoculated plants respectively.

5. Effect of different inoculum levels of *F. oxysporum* on plant growth, number of pods, chlorophyll contents, nodulation and wilt index:

It is clear from Table 4.a & b that plant growth of *G. max* reduced with on increase in fungus *F. oxysporum* inoculum level to a varying degree. The root length of plant was 35.00, 29.11,

Table-4.a: Effect of different inoculum levels of *Fusarium oxysporum* on plant growth and number of pods on *Glycine max* var. L.

Treatments	Length (cm)				Fresh wt. (g)				Dry wt. (g)				No. of Pods	% Decrease
	Shoot	Root	Total	% Decrease	Shoot	Root	Total	% Decrease	Shoot	Root	Total	% Decrease		
Control (uninoculated) F. oxysporum	44.50	41.10	85.60	-	23.00	12.00	35.00	-	8.00	1.801	9.80	-	25.33	-
0.25(g)	39.10	35.00	74.10	13.43	18.51	8.50	27.01	22.83	5.82	0.903	6.72	31.43	20.00	21.04
0.50	33.23	29.11	62.34	27.16	16.00	7.52	23.52	32.80	5.00	0.701	5.70	41.84	17.33	31.58
1.00	27.00	22.00	49.00	42.76	14.03	6.31	20.34	41.89	4.27	0.422	4.69	52.14	12.67	49.98
2.00	22.08	16.01	38.09	55.50	10.00	5.07	15.07	56.94	3.25	0.252	3.50	64.29	9.33	63.17
CD [P(0.05)]			4.91				2.59				0.85		1.42	
CD [P(0.01)]			7.14				3.77				1.24		2.06	

Table-4.b: Effect of different inoculum levels of *Fusarium oxysporum* on chlorophyll contents, nematode development, nodulation and gall formation.

Treatments	Chl.(a) 'mg/g'	% decrease	Chl.(b) 'mg/g'	% decrease	Total Chl. (a+b) 'mg/g'	% decrease	Nodule/root system		Wilt index
							Number	% decrease	
Control (uninoculated)	1.200	-	1.501	-	2.701	-	75.33	-	-
F. oxysporum									
0.25(g)	1.050	12.50	1.140	24.05	2.190	18.92	50.67	32.74	1.30
0.50	0.710	40.83	0.840	44.04	1.550	42.61	44.33	41.15	2.00
1.00	0.441	63.25	0.500	66.69	0.941	65.16	38.33	49.12	3.00
2.00	0.305	74.58	0.360	76.02	0.665	75.38	32.67	56.63	3.60
CD [P(0.05)]	0.009		0.017		0.025		7.53		0.61
CD [P(0.01)]	0.013		0.024		0.036		10.96		0.89

22.00 and 16.01cm. as compared to 41.10cm in uninoculated control and shoot length of plant was recorded by 39.10, 33.23, 27.00 and 22.08cm as compared to 44.50cm in uninoculated in 0.25, 0.50, 1.00 and 2.00g mycelium of *F. oxysporum* inoculated plants respectively. Reduction in total plant length was recorded by 13.43, 27.16,42.76 and 55.50% over control in above given treatments respectively.

The fresh weight of root was recorded by 8.50, 7.52, 6.31 and 5.07g as compared to 12.00g in uninoculated control and fresh weight of shoot was 18.51, 16.00, 14.03 and 10.00g as compared to 23.00g in uninoculated control in 0.25, 0.50, 1.00 and 2.00g mycelium of *F. oxysporum* inoculated plants respectively. Reduction in total plant fresh weight was recorded by 22.83, 32.80, 41.89 and 56.94% over control in above given treatments respectively.

The dry weight of root was recorded by 0.903, 0.701, 0.422 and 0.252g as compared to 1.801g in uninoculated control and dry weight of shoot was 5.82. 5.00, 4.27 and 3.25g as compared to 8.00g in uninoculated control in 0.25, 0.50, 1.00 and 2.00g mycelium of *F. oxysporum* inoculated plants respectively. Reduction in total plant dry weight was recorded by

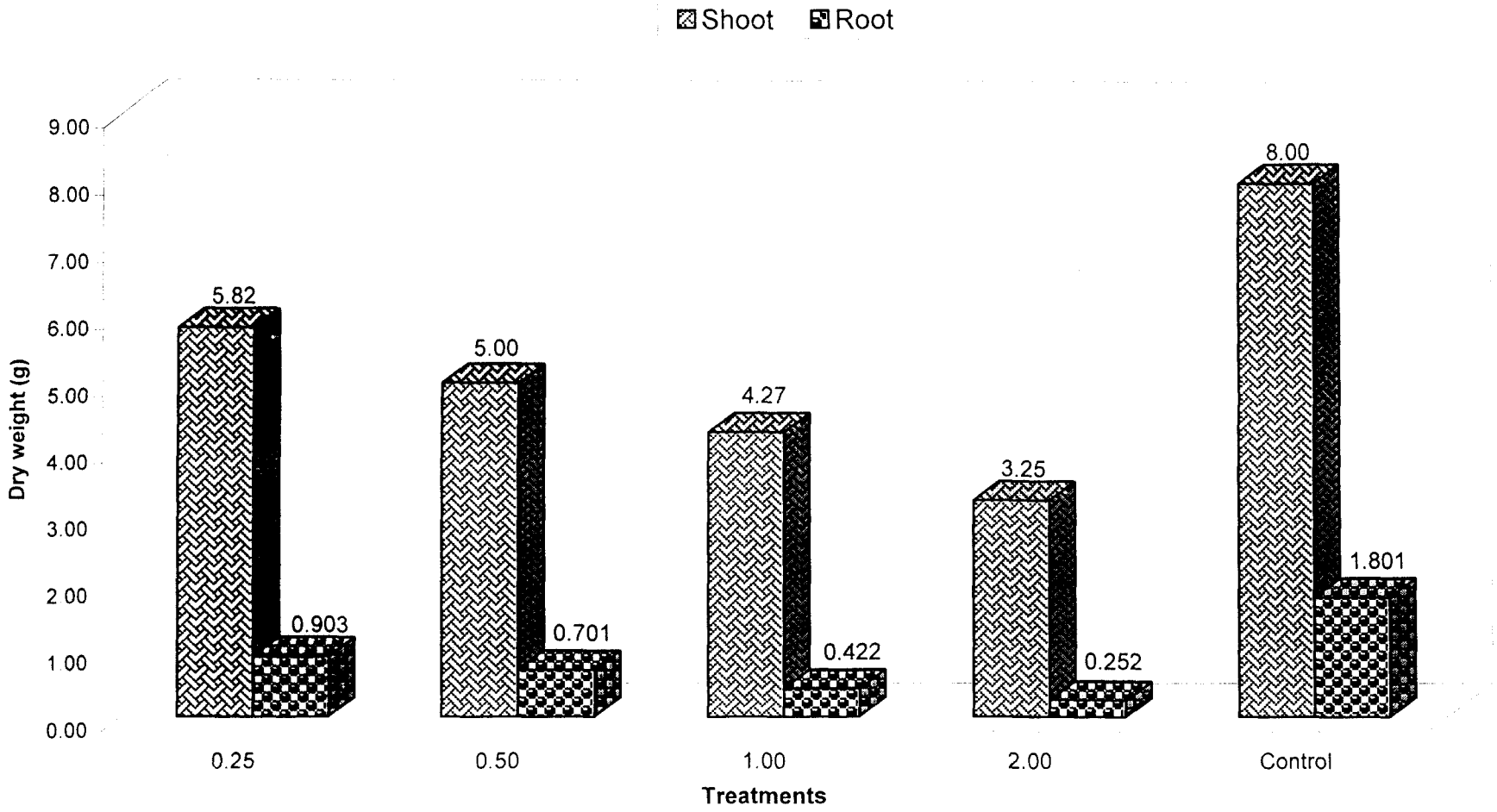

Fig. 4.a: Effect of different inoculum levels of *Fusarium oxysporum* on dry weight of shoot and root.

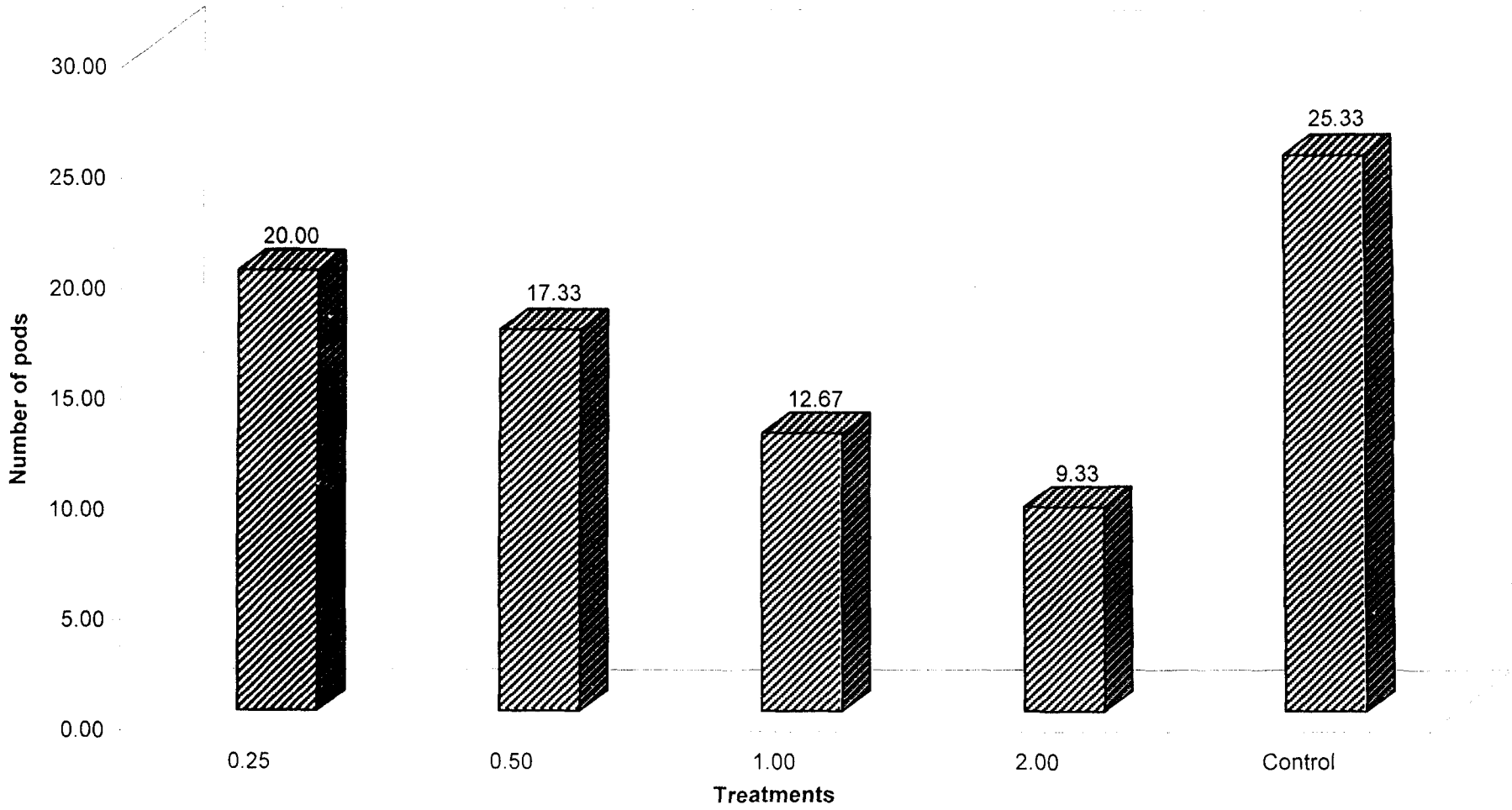

Fig. 4.a: Effect of different inoculum levels of *Fusarium oxysporum* on number of pods.

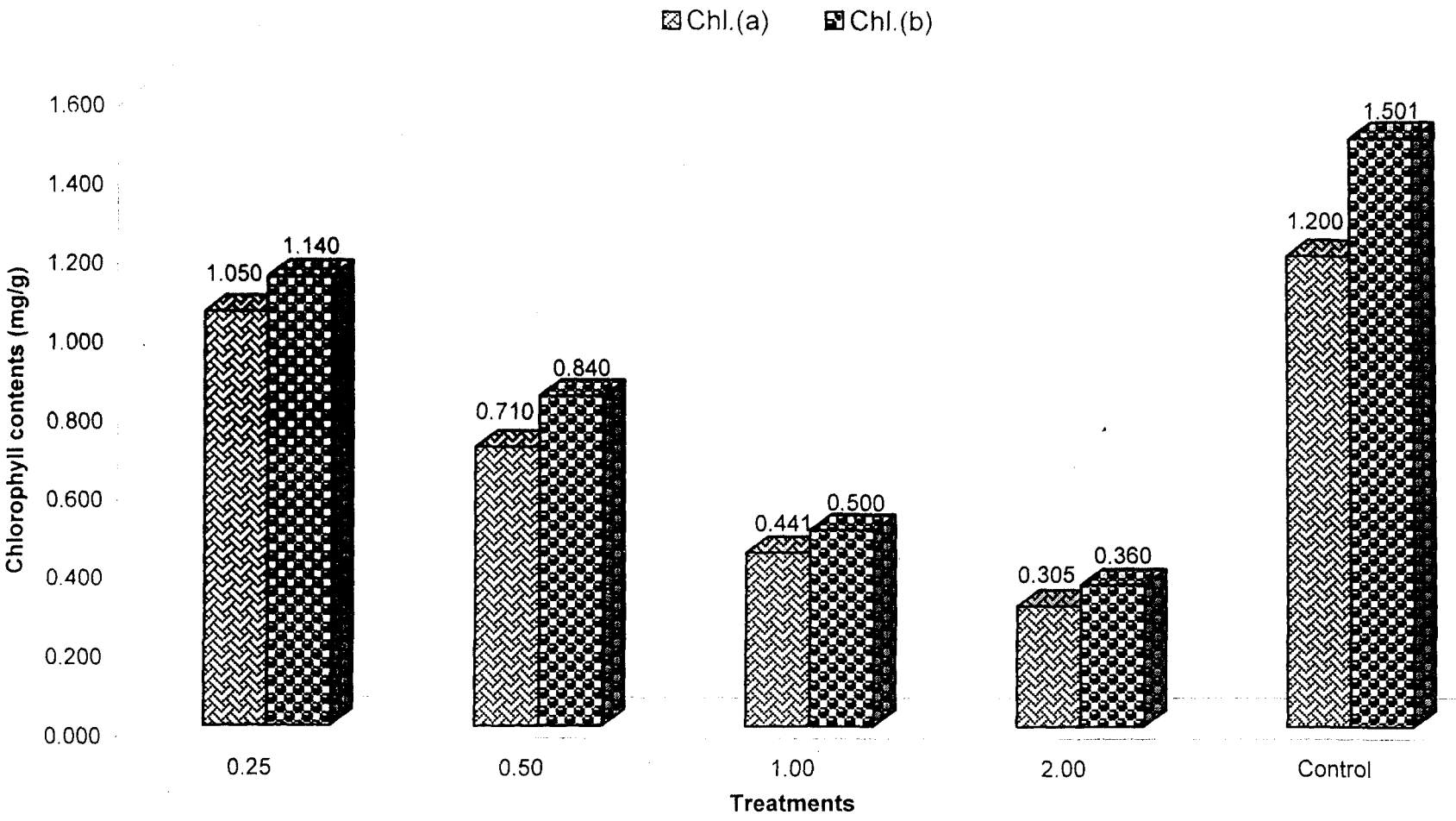

Fig. 4.b: Effect of different inoculum levels of *Fusarium oxysporum* on chlorophyll contents.

31.43, 41.84, 52.14 and 64.29% over control in above given treatments respectively.

Total number of pods per plant was recorded by 20.00, 17.33, 12.67 and 9.33 as compared to 25.33 in uninoculated control in 0.25, 0.50, 1.00 and 2.00g mycelium of *F. oxysporum* inoculated plants respectively. Reduction in total number of pods was 21.04, 31.58, 49.98 and 63.17% over control in above given treatments respectively.

Chlorophyll 'a' was recorded by 1.050, 0.710, 0.441 and 0.305 mg/g, as compared to 1.200 in uninoculated control in 0.25, 0.50, 1.00 and 2.00g mycelium of *F. oxysporum* inoculated plants respectively. Reduction in chlorophyll 'a' was 12.50, 40.83, 63.25 and 74.58% over control in above given treatments respectively.

Chlorophyll 'b' was recorded by 1.140, 0.8.40, 0.500 and 0.360 mg/g as compared to 1.501 mg/g in uninoculated control in 0.25, 0.50, 1.00 and 2.00g mycelium of *F. oxysporum* inoculated plants respectively. Reduction in chlorophyll 'b' was 24.05, 44.04, 66.69 and 76.02% over control in above given treatments respectively.

Total chlorophyll (a+b) was recorded by 2.190, 1.550, 0.941 and 0.665 mg/g as compared to 2.701 in uninoculated

control in 0.25, 0.50, 1.00 and 2.00g mycelium of *F. oxyspurum* inoculated plants respectively. Reduction in total chlorophyll (a+b) was 18.92, 42.61, 65.16 and 75.38% over control in above given treatments respectively.

Total number of nodules per root system were recorded by 50.67, 44.33, 38.33 and 32.67 as compared to 75.33 in uninoculated control in treatments 0.25, 0.50, 1.00 and 2.00 g mycelium of *F. oxysporum* inoculated plants respectively. Reduction in nodules per root system was recorded by 32.74, 41.15, 49.12 and 56.63% over control in above given treatments respectively.

The wilt index was observed 1.30, 2.00, 3.00 and 3.60 when 0.25, 0.50, 1.00 and 2.00g mycelium of *Fusarium oxysporum* was inoculated respectively.

6. Effect of individual, sequential and simultaneous inoculation of *M. incognita, P. aphanidermatum and F. oxysporum* on growth, number of pods, chlorophyll content, nematode multiplication, gall formation, nodulation root-rot and wilt development on *Glycine max*:

It is evident from the data given in Table 5.a & b that the root length of plant was 31.02, 28.00, 25.01, 24.20, 22.10,

Table-5.a: Effect of individual, sequential and simultaneous inoculation of *Meloidogyne incognita, Pythium aphanidermatum* and *Fusarium oxysporum* on plant growth and number of pods on *Glycine max* var. L.

Treatments	Length (cm)				Fresh wt. (g)				Dry wt. (g)				No. of Pods	% Decrease
	Shoot	Root	Total	% Decrease	Shoot	Root	Total	% Decrease	Shoot	Root	Total	% Decrease		
Control (uninoculated)	45.00	42.00	87.00	-	24.00	13.50	37.50	-	9.00	1.904	10.90	-	26.33	-
M. incognita	34.01	31 02	65.03	25.25	17.07	9.05	26.12	30.35	7.00	1.053	8.05	26.15	18.67	29.09
P.aphanidermatum	31.00	28.00	59.00	32.18	15.70	8.20	23.90	36.27	6.20	1.003	7.20	33.94	17.33	34.18
F. oxysporum	28.52	25.01	53.53	38.47	14.00	7.30	21.30	43.20	5.71	0.951	6.66	38.90	14.00	46.83
Pa→Mi	28.00	24.20	52.20	40.00	13.20	6.63	19.83	47.12	5.20	0.822	6.02	44.77	13.67	48.08
Fo→Mi	26.00	22.10	48.10	44.71	12.10	5.40	17.50	53.33	4.40	0.704	5.10	53.21	11.67	55.68
Fo+Pa	25.03	21.10	46.13	46.98	11.30	4.80	16.10	57.07	3.90	0.611	4.51	58.62	10.67	59.48
Mi→Pa	23.00	19.60	42.60	51.03	10.28	4.59	14.87	61.25	3.30	0.572	3.87	64.50	9.00	65.82
Mi→Fo	21.50	18.10	39.60	54.60	9.00	4.00	13.00	65.33	2.81	0.541	3.35	69.27	8.00	69.62
Mi+Pa.	20.00	16.30	36.30	58.28	8.10	3.20	11.30	69.87	2.42	0.503	2.92	73.21	6.67	74.67
Mi+Fo	18.03	14.00	32.03	63.18	6.82	2.60	9.42	74.88	1.70	0.394	2.09	80.83	5.33	79.76
Mi+Fo+Pa	16.00	12.50	28.50	67.24	5.40	2.00	7.40	80.27	1.00	0.303	1.30	88.07	4.33	83.55
CD [P(0.05)]			2.14				1.60				0.68		0.89	
CD [P(0.01)]			2.91				2.18				0.93		1.19	

Table-5.b: Effect of individual, sequential and simultaneous inoculation of *Meloidogyne incognita, Pythium aphanidermatum* and *Fusarium oxysporum* on chlorophyll contents, nodulation, gall formation, root-rot, wilt index and nematode development.

Treatments	Chl. (a) 'mg/g'	% decrease	Chl (b) 'mg/g'	% decrease	Total Chl (a+b) 'mg/g'	% decrease	Nodule/root system		Galls/root system		Root-rot index	Wilt index	Nematode population			Nematode multiplication Rf=pf/pi	% decrase
							Number	% decrease	Number	% decrease			Tissue	Soil	Total		
Control (uninoculated)	1.230	-	1.560	-	2.790	-	79.67	-	-	-	-	-	-	-	-	-	-
M. incognita	0.670	45.53	0.990	36.54	1.660	40.50	56.33	29.30	94	-	-	-	15893.7	128.3	16022	16.02	-
P.aphanidermatum	0.601	51.14	0.870	44.23	1.471	47.28	50.33	36.83	-	-	2.00	-	-	-	-	-	-
F. oxysporum	0.550	55.28	0.780	50.00	1.330	52.33	42.33	46.87	-	-	-	3.00	-	-	-	-	-
Pa→Mi	0.480	60.98	0.690	55.77	1.170	58.06	39.67	50.21	51	45.74	2.35	-	8566.3	84.7	8651	8.65	46.00
Fo→Mi	0.375	69.51	0.570	63.46	0.945	66.13	35.67	55.23	46	51.06	-	3.35	7453.3	72.7	7526	7.52	53.06
Fo+Pa	0.350	71.54	0.500	67.95	0.850	69.53	30.33	61.93	-	-	2.10	3.10	-	-	-	-	-
Mi→Pa	0.295	76.02	0.442	71.67	0.737	73.58	28.33	64.44	80	14.89	2.90	-	14702.8	108.2	14811	14.81	7.55
Mi→Fo	0.245	80.08	0.382	75.51	0.627	77.53	25.67	67.78	71	24.47	-	3.70	13981.4	104.6	14086	14.08	12.07
Mi+Pa	0.210	82.93	0.301	80.71	0.511	81.68	20.33	74.78	64	31.91	3.00	-	12317.9	87.1	12405	12.40	22.57
Mi+Fo	0.150	87.80	0.235	84.94	0.385	86.20	14.67	81.59	56	40.43	-	3.85	9793.2	56.8	9850	9.85	38.51
Mi+Fo+Pa	0.110	91.06	0.181	88.40	0.291	89.57	9.33	88.23	36	61.70	3.10	4.00	8102.4	40.6	8143	8.14	49.17
CD [P(0.05)]	0.014		0.019		0.032		8.17		8.45		0.31	0.22				0.14	
CD [P(0.01)]	0.019		0.026		0.044		11.14		11.48		0.42	0.30				0.19	

21.10, 19.60, 18.10, 16.30, 14.00 and 12.50cm as compared to 42.00cm in uninoculated control and shoot length of plant 34.01, 31,00, 28.52, 28.00, 26.00, 25.03, 23.00, 21.50, 20.00, 18.03 and 16.00cm as compared to 45.00cm in uninoculated control in Mi, Pa, Fo, Pa→Mi, Fo→Mi, Fo+Pa, Mi→Pa, Mi→Fo, Mi+Pa, Mi+Fo, and Mi+Pa+Fo treatments respectively. Reduction in plant length was 25.25, 32.18, 38.47, 40.00, 44.71, 46.98, 51.03, 54.60, 58.28, 63.18 and 67.24% over control in above given treatments respectively.

Fresh weight of root was recorded by 9.05, 8.20, 7.30, 6.63, 5.40, 4.80, 4.59, 4.00, 3.20, 2.60 and 2.00g as compared to 13.50g in uninoculated control and fresh weight of shoot was 17.07, 15.70, 14.00, 13.20, 12.10, 11.30, 10.28, 9.00, 8.10, 6.82 and 5.40g as compared to 24.00g in uninoculated control in Mi, Pa, Fo, Pa→Mi, Fo→Mi, Fo+Pa Mi→Pa, Mi→Fo, Mi+Pa, Mi+FO, and Mi+Pa+Fo treatments respectively. Reduction in total plant fresh weight was recorded by 30.35, 36.27, 43.20, 47.12, 53.33, 57.07, 61.25, 65.33, 69.87, 74.88 and 80.27% over control in above given treatments respectively.

Dry weight of root was recorded by 1.053, 1.003 0.951, 0.822, 0.704, 0.611, 0.572, 0.541, 0.503, 0.394, and 0.303g

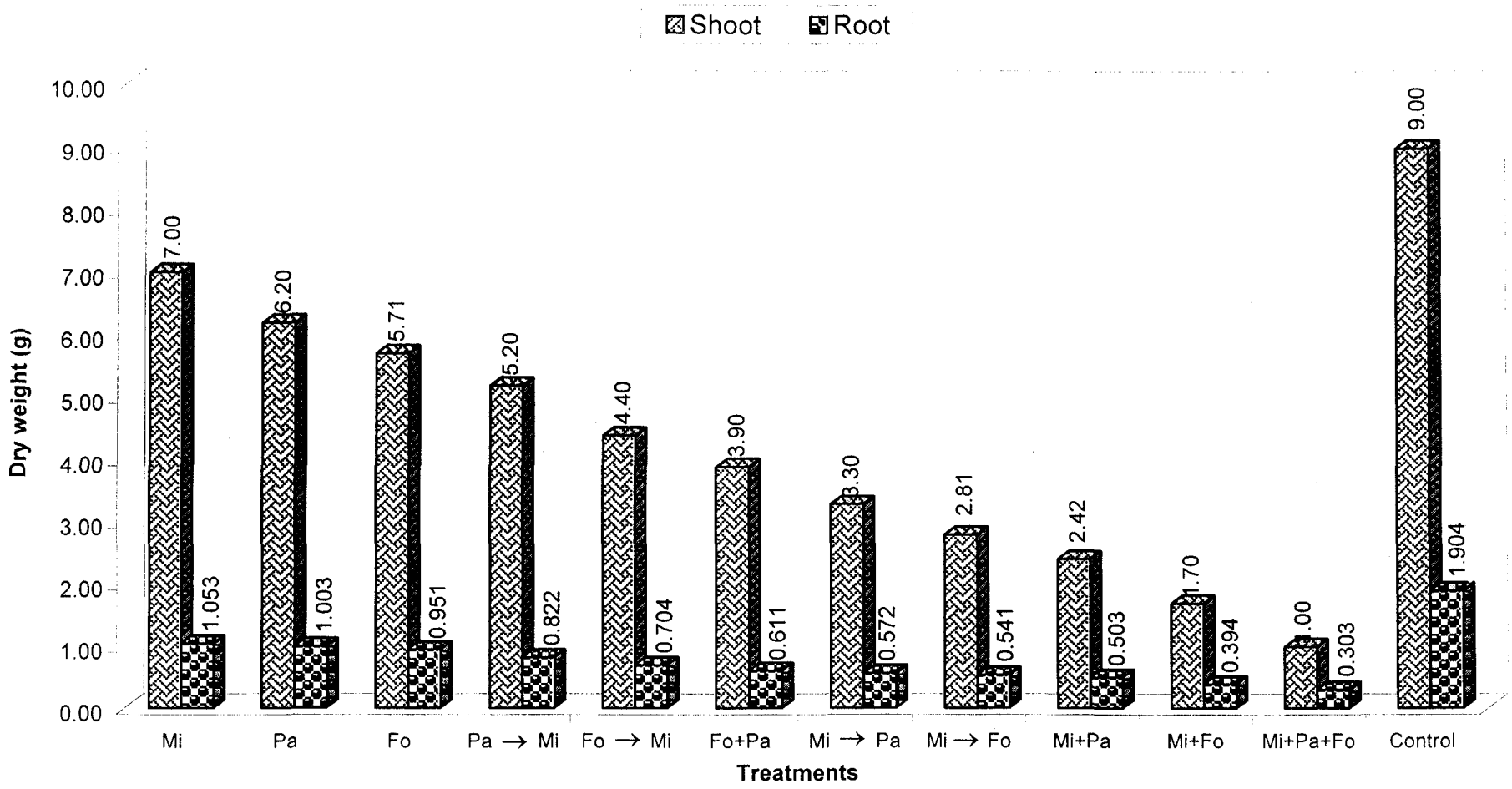

Fig. 5.a: Effect of individual, sequential and simultaneous inoculation of *Meloidogyne incognita*, *Pythium aphanidermatum* and *Fusarium oxysporum* on dry weight.

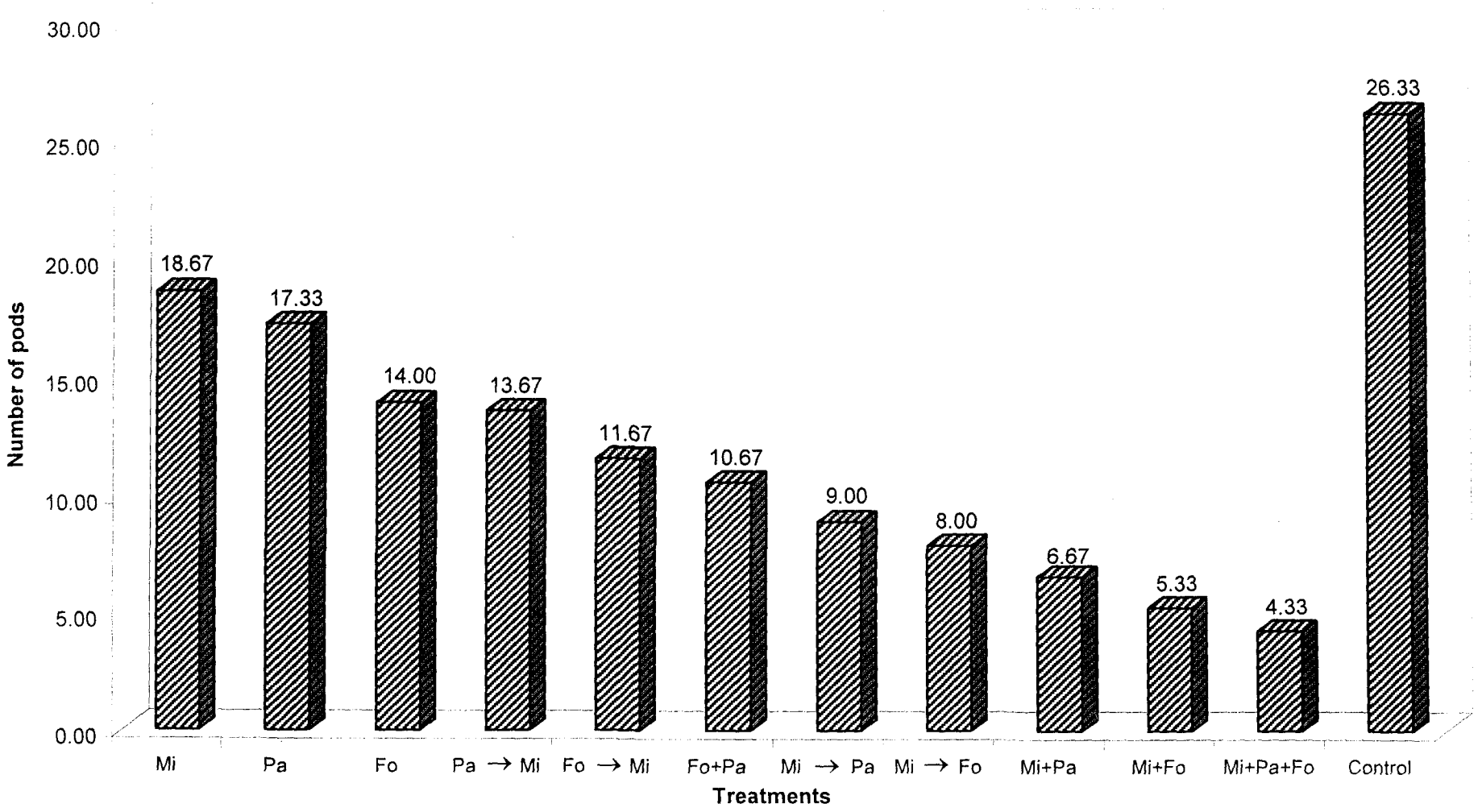

Fig. 5.a: Effect of individual, sequential and simultaneous inoculation of *Meloidogyne incognita*, *Pythium aphanidermatum* and *Fusarium oxysporum* on number of pods.

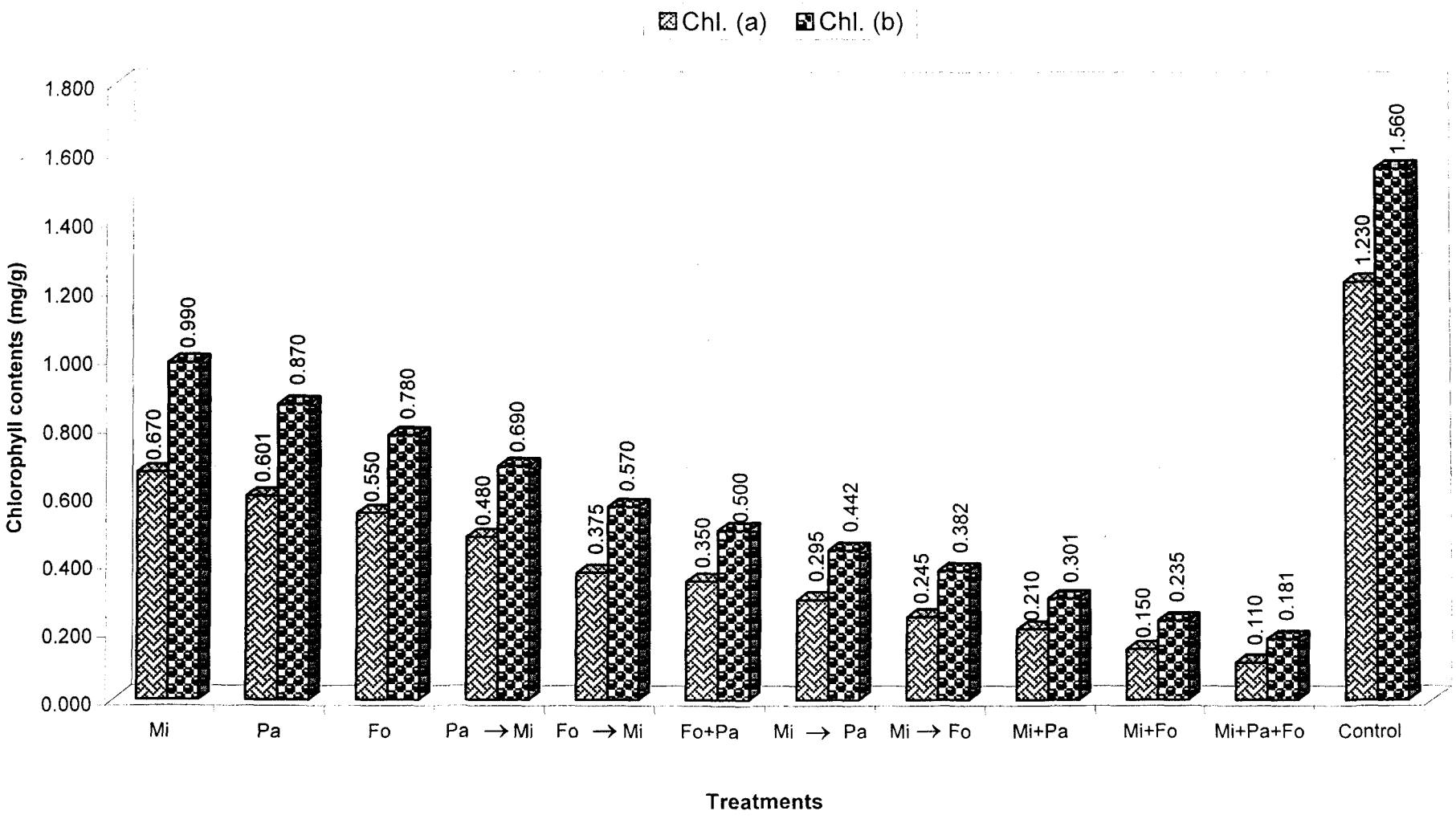

Fig. 5.b: Effect of individual, sequential and simultaneous inoculation of *Meloidogyne incognita*, *Pythium aphanidermatum* and *Fusarium oxysporum* on chlorophyll contents.

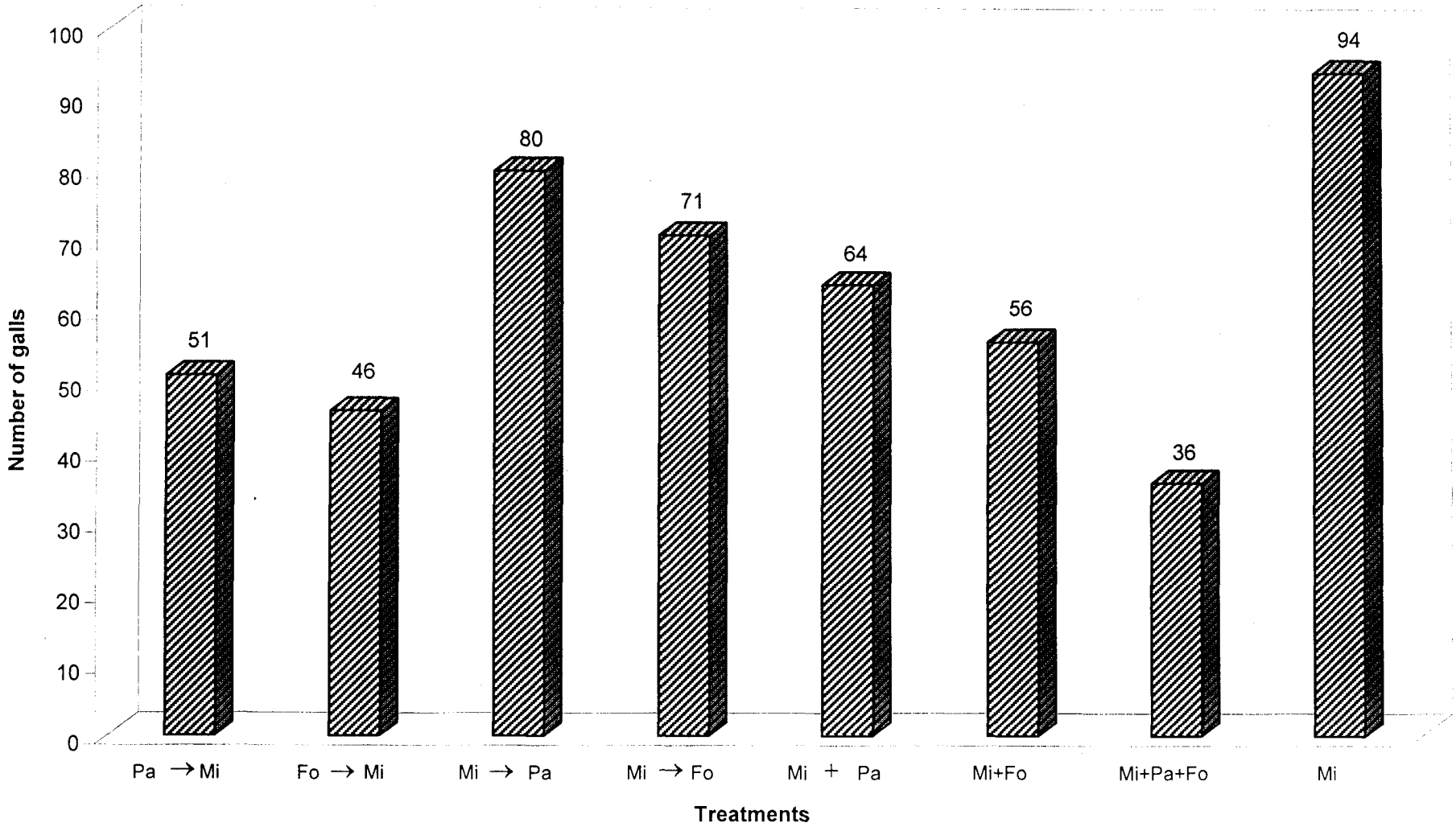

Fig. 5.b: Effect of individual, sequential and simultaneous inoculation of *Meloidogyne incognita*, *Pythium aphanidermatum* and *Fusarium oxysporum* on gall formation.

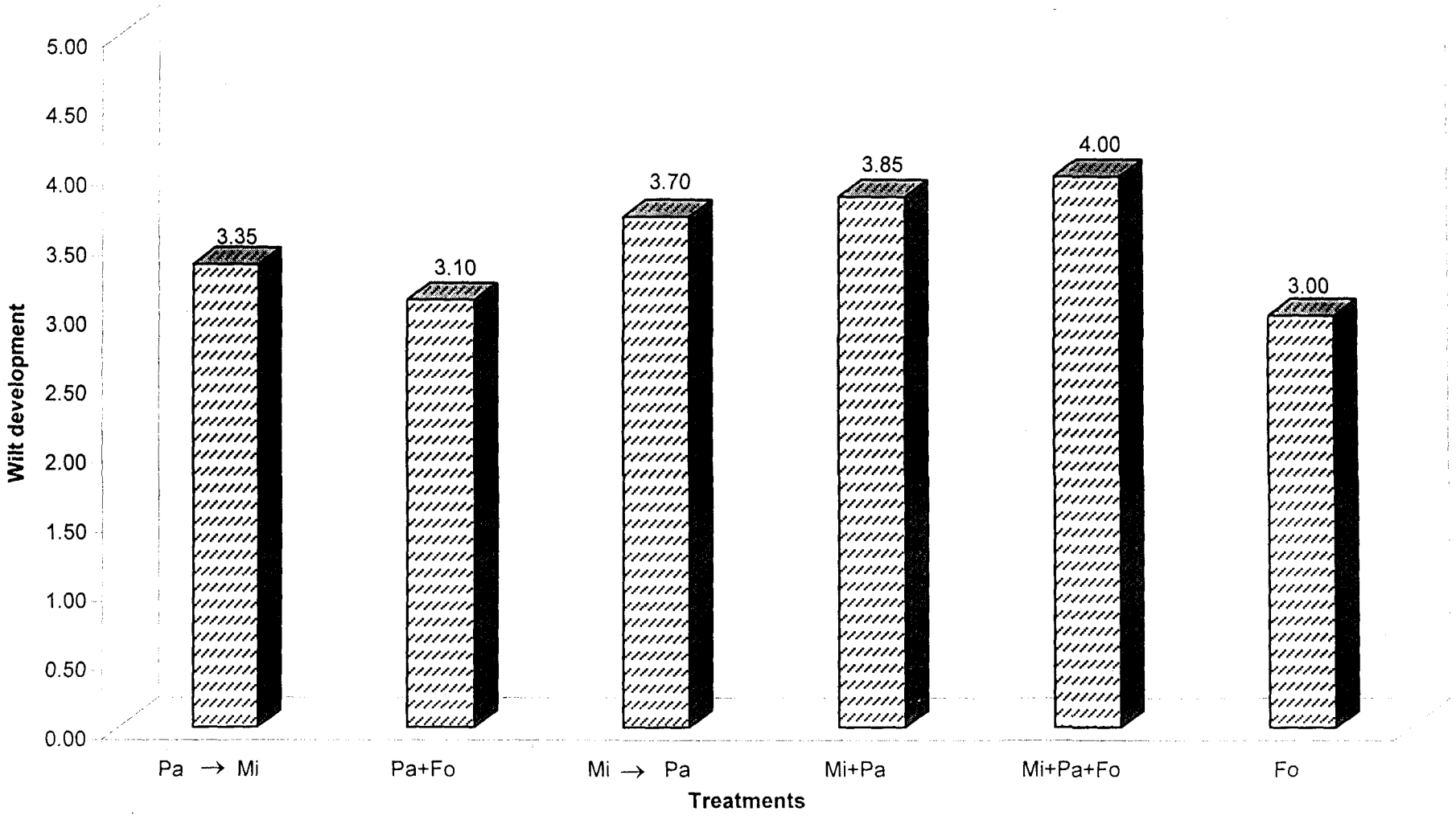

Fig. 5.b: Effect of individual, sequential and simultaneous inoculation of *Meloidogyne incognita*, *Pythium aphanidermatum* and *Fusarium oxysporum* on wilt development.

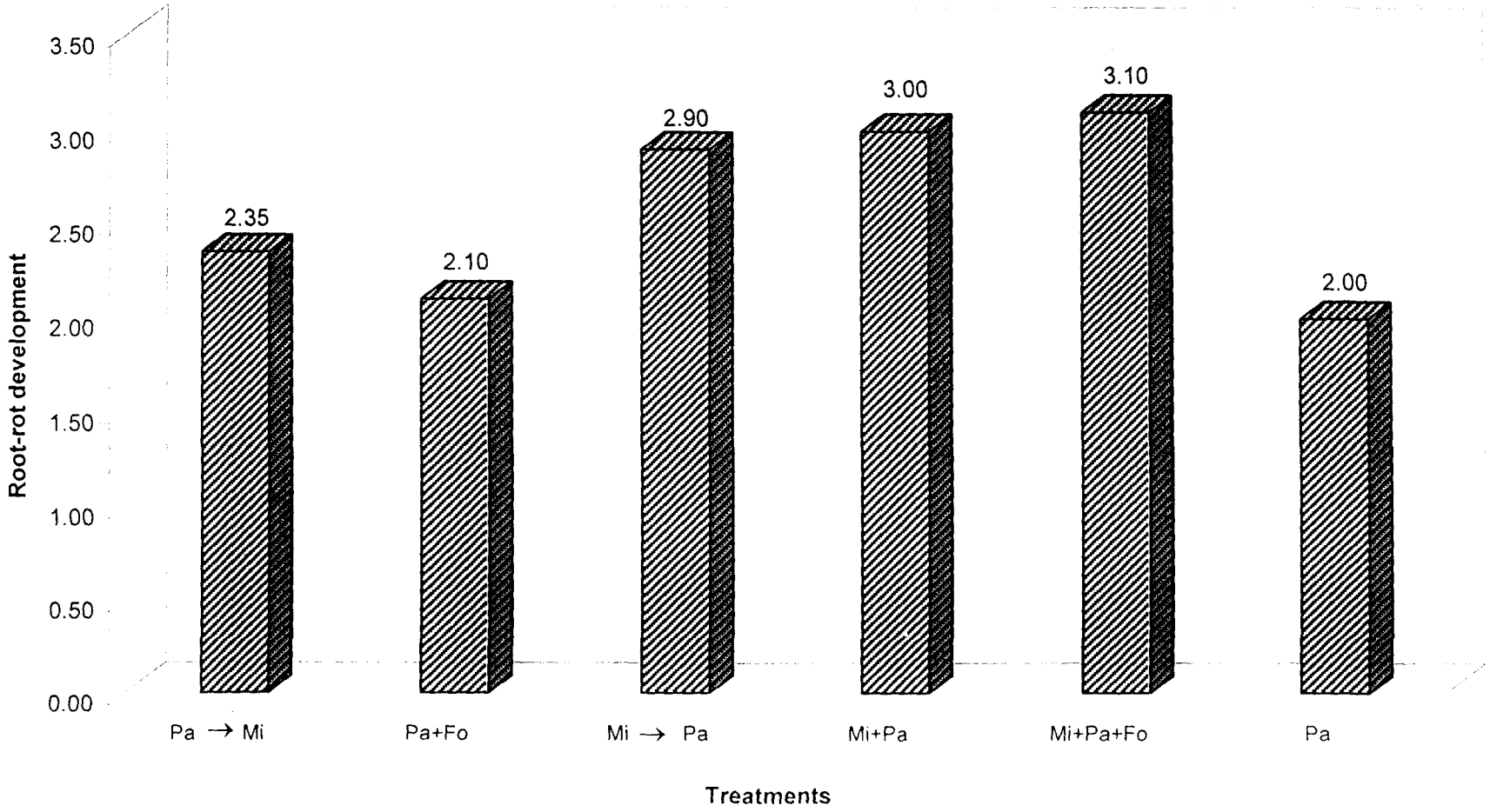

Fig. 5.b: Effect of individual, sequential and simultaneous inoculation of *Meloidogyne incognita, Pythium aphanidermatum* and *Fusarium oxysporum* on root-rot.

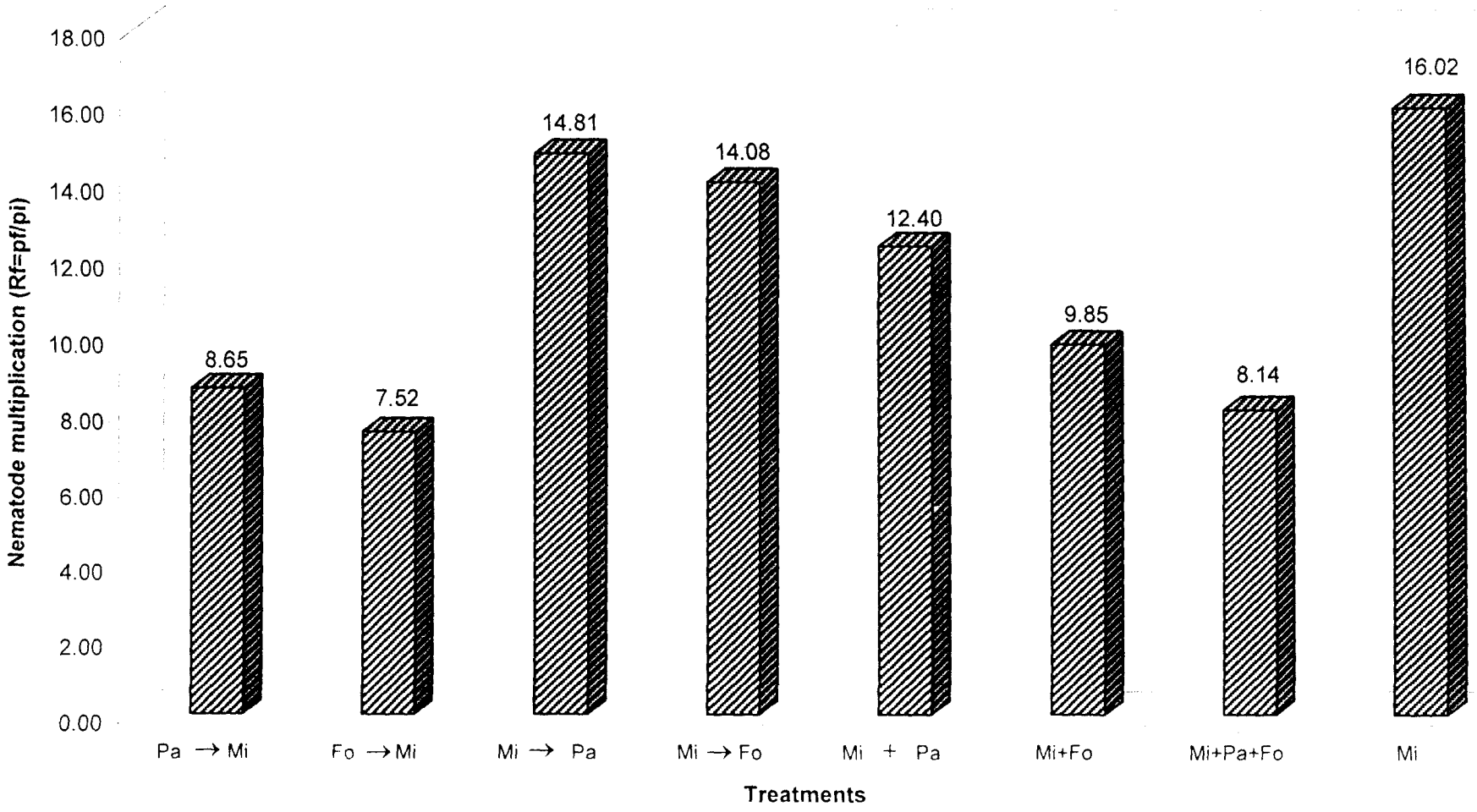

Fig. 5.b: Effect of individual, sequential and simultaneous inoculation of *Meloidogyne incognita*, *Pythium aphanidermatum* and *Fusarium oxysporum* on nematode multiplication.

as compared to 1.904g in uninoculated control and dry weight of shoot was 7.00, 6.20, 5.71, 5.20, 4.40, 3.90, 3.30, 2.81, 2.42, 1.70 and 1.00g as compared to 9.00g in uninoculated control in Mi, Pa, Fo, Pa→Mi, Fo→Mi, Fo+Pa, Mi→Pa, Mi→Fo, Mi+Pa, Mi+Fo, and Mi+Pa+Fo treatments respectively. Reduction in total plant dry weight was 26.15, 33.94, 38.90, 44.77, 53.21, 58.62, 64.50, 69.27, 73.21, 80.83 and 88.07% over control in above given treatments respectively.

Total number of pods per plant were recorded by 18.67, 17.33, 14.00, 13.67, 11.67, 10.67, 9.00, 8.00, 6.67, 5.33 and 4.33 as compared to 26.33 in uninoculated control in Mi,Pa, Fo, Pa→Mi, Fo→Mi, Fo+Pa, Mi.→ Pa, Mi→Fo, Mi+Pa, Mi+Fo, and Mi+Pa+ Fo treatments respectively. Total number of pods per pant were reduced by 29.09, 34.18, 46.83, 48.08, 55.68, 59.48, 65.82, 69.62, 74.67, 79.76 and 83.55% over control in above given treatments respectively.

Chlorophyll 'a' was recorded by 0.670, 0.601, 0.550, 0.480, 0.375, 0.350, 0.295, 0.245, 0.210, 0.150 and 0.110mg/g as compared to 1.230 mg/g in uninoculated control in Mi, Pa, Fo, Pa→Mi, Fo→Mi, Fo+Pa, Mi→Pa, Mi→Fo, and Mi+Pa+Fo treatments respectively. Reduction in chlorophyll 'a' was

recorded by 45.53, 51.14, 55.28, 60.98, 69.51, 71.54, 76.02, 80.08, 82.93, 87.80 and 91.06% over control in above given treatments respectively.

Chlorophyll 'b' was recorded by 0.990, 0.870, 0.780, 0.690, 0.570, 0.500, 0.442, 0.382, 0.301, 0.235 and 0.181 mg/g as compared to 1.560 mg/g in uninoculated control in Mi, Pa, Fo, Pa→Mi, Fo→Mi, Fo+Pa, Mi→Pa, Mi→Fo, and Mi+Pa+Fo treatments respectively. Reduction in chlorophyll 'b' was 36.54, 44.23, 50.00, 55.77, 63.46, 67.95, 71.67, 75.51, 80.71, 84.94 and 88.40% over control in above given treatments respectively.

Total chlorophyll (a+b) was recorded by 1.660, 1.471, 1.330, 1.170, 0.945, 0.850, 0.737, 0.627, 0.511, 0.385 and 0.291 mg/g as compared to 2.790mg/g in uninoculated control in Mi,Pa, Fo, Pa→Mi, Fo→Mi, Fo+Pa, Mi→Pa, Mi→Fo, Mi+Pa, Mi+Fo, and Mi+Pa+Fo treatments respectively. Reduction in total chlorophyll (a+b) was recorded by 40.50, 47.28, 52.33, 58.06, 66.13, 69.53, 73.58, 77.53, 81.68, 86.20 and 89.57% over control in above given treatments respectively.

Total number of nodules per root system were recorded by 56.33, 50.33, 42.33, 39.67, 35.67, 30.33, 28.33, 25.67,

20.33, 14.67 and 9.33 as compared to 79.67 in uninoculated control in Mi,Pa, Fo, Pa→Mi, Fo→Mi, Pa+Fo, Mi→Pa, Mi→Fo, Mi+Pa, Mi+Fo and Mi+Pa+Fo treatments respectively. Reduction in total number of nodules per root system was 29.30, 36.83, 46.87, 50.21, 55.23, 61.93, 64.44, 67.78, 74.48, 81.59 and 88.23% over control in above given treatments respectively.

Rate of nematode multiplication was 8.65, 7.52, 14.81, 14.08, 12.40, 9.85 and 8.14 as against 16.02 in nematode alone (Mi) control in the following treatments Pa→Mi, Fo→Mi, Mi→Pa, Mi→Fo, Mi+Pa, Mi+Fo, and Mi+Pa+Fo respectively. The reduction in nematode multiplication was 46.00, 53.06, 7.55, 12.07, 22.57, 38.51 and 49.17% over control (Mi) in above given treatments respectively.

Total root galls per root system were recorded by 51, 46.80 71, 64, 56 and 35 as compared to 94 in nematode alone control (Mi) in Pa→Mi, Fo→Mi, Mi→Pa, Mi→Fo, Mi→Pa, Mi→Fo, and Mi+Pa+Fo treatments respectively. Galls/roots system were decreased by 45.74, 51.06, 14.89, 24.47, 31.91, 40.43 and 61.70% over control in above given treatments respectively.

Total root-rot index was observed 2.35, 2.10, 2.90, 3.00 and 3.10 as compared to 2.00 in plants inoculated with *Pythium*

aphanidermatum alone in above Pa→Mi, Fo+Pa, Mi→Pa, Mi→Pa, and Mi+Pa+Fo treatments respectively.

Wilt-index was recorded by 3.35, 3.10, 3.70, 3.85 and 4.00 as compared to 3.00 in plants inoculated with *Fusarium oxysporum* alone in Fo→Mi, Fo+Pa, Mi→Fo, Mi+Fo, and Mi+Pa+Fo treatments respectively.

Discussion

DISCUSSION

The different concentrations of culture filtrates of *Fusarium oxysporum* and *Pythium aphanidermatum* significantly inhibited the hatching of root-knot nematode, *Meloidogyne incognita* to a varying degree. Larval emergence was lowest in highest concentration S and it was low upto S/10 concentrations of both the fungi while it was highest in lowest concentration S/1000. Larval emergence was inversely proportional to filtrate concentrations.

Similarly the percent larval mortality differs with different concentrations of culture filtrates of two fungi. Mortality of larvae is directly proportional to the concentrations of culture filtrates and duration of exposure. The culture filtrate of *Fusarium oxysporum* was highly toxic to nematode larvae followed by *Pythium aphanidermatum*, as mortality was 100 percent in S concentration of *F. oxysporum* after 72 hours of exposures.

An increase in larval mortality and reduction in hatching of root-knot nematode, *M. incognita* may be due to the effect of antagonistic fungal metabolites like oxalic acid, fusaric acid etc. released in cultural filtrates which might be toxic to nematodes (Manku, 1969; Shukla and Sawarup, 1971; Desai et al., 1972;

Azam, 1975; Azam et al., 1979; Khan & Khan 1992 and Khan 1999. Our results are in conformity with above workers.

Soybean *(G. max)* seedlings were inoculated with different inoculum levels of root-knot nematodes, *M. incognita* (250, 500, 1000 and 2000 juveniles per plant) and the fungi *P. aphanidermatum* and *F. oxysporum* (0.25, 0.50 1.00 and 2.00g per plant) separately. Gradual reduction in plant growth was recorded by increased inoculum levels of all the pathogens. When 1000 or more nematodes or 1.0g or more fungus was inoculated per plant then there was a significant reduction in total plant weight. Increasing inoculum levels of *M. inocgnita* (250, 500, 1000 and 2000J_2) caused more reduction in total plant weight, nodulation, number of pods and chlorophyll contents. Rate of nematode multiplication decreased as the inoculum levels of nematode increased, may be due to competition for food and space. Surface area of root remained same for both lower and higher inoculum levels. Overcrowding of nematodes at higher inoculum density created competition among the nematodes which resulted in their natural death and reduced multiplication. In case of low inoculum level, multiplication rate becomes high due to abundance of food, space and less competition among them. Root galls increased as inoculum levels increased. Our results are in

conformity with those of Sharma and Radriguez (1982) and Meena and Mishra (1994). An increase in inoculum levels of *P. aphanidermatum* and *F. oxysporum* caused greater root-rot and wilting and resulted a decrease in plant growth and nodulation. *Fusarium* spp. produced fusaric acid which is known to cause stunting, chlorosis and even death of infected plants. (Yabuta et al, 1934; Subba Rao, 1957; Andal, 1957).

In combined inoculations, highest reduction was recorded in total plant weight, number of pods, chlorophyll contents nodulation, when plants were inoculated with all the three pathogens simultaneously. The plant damage was higher when nematode inoculated 15 days prior to fungus than that of reciprocal treatment. The highest reduction in plant growth and nodulation was in simultaneous inoculation in contrast to sequential inoculations (Azam, 1975, and Mani, 1983), because each pathogen, has equal opportunity to parasitize the roots and alter the morphology, anatomy and biochemistry of the host plant (Young et al., 1976; Mani 1983) while in remaining treatments there was lack of time (15 days) and pathogens have less time to parasitize on host plants, that's why there was less reduction in plant growth and nodulation as compared to simultaneous inoculation. In sequential inoculations another factor may be that

the seedlings have progressed to such a stage of their development that they no longer remain susceptible to one of the three pathogens. The results are in agreement with those of Azam (1975) and Azam et al., (1984). *M. incognita* multiplication and galling was affected in all combinations and with the fungus and it is due to the inhibitory effect of fungus on nematodes as compared to nematode present alone. The reduction in nematode multiplication and galling was also significantly high when fungus was inoculated prior to nematode and least in its vice-versa treatments.

Root-rot and wilting increased in presence of nematode due to mobilization of nutrients to infected roots which benefited the fungal growth and increased the disease incidence (Azam et al., 1977). In treatments where fungus was inoculated earlier than nematodes the fungus produced certain metabolites which adversely affect the nematode feeding sites by damaging the cells there by resulting a reduction in nematode development and less root-knot galls formation. Whereas, in prior establishment of nematodes, the nematode predisposed the roots in advance to fungal attack by inducing the physiological and biochemical changes resulting in the aggressive behaviour of the fungus (Siddiqui, 1990).

Significant reduction was also observed in the photosynthetic pigment chlorophyll due to these pathogens. Decrease in chlorophyll contents in infected plants, adversely affect the photosynthesis which in turn impede development of plants in terms of reduced plant weight, number of flowers and delayed flowering, ultimately resulting in reduced yield (Melakeberhan et al., 1985) imbalance in the translocation process (Bird and Loveys, 1975; McClure, 1977; Melakeberhan et al., 1985) reduced production and supply of carbohydrates to nodules for carrying out nitrogen fixation (Chahal and Chahal, 1987; Anwar and Alam, 1989; Tiyagi and Alam, 1990; Chandel et al., 1993; Vashisth et al., 1994) and reduction in chlorophyll contents due to fungus (Murumkar and Chawan, 1985). Significantly there was higher reduction in chlorophyll contents (chl. a,b and total chl.) in concomitant inoculations than in either of them alone (Tiyagi 1990). Our results are in conformity with those workers given above.

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